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A FAMILY OUTBREAK OF HISTOPLASMOSIS

I. CLINICAL, LABORATORY, AND FOLLOW-UP STUDIES

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THIS report is concerned with an outbreak of histoplasmosis in a family of 4 persons living in Montgomery County, Maryland, within 5 miles of the city limits of Washington, D. C. As far as can be determined it is the first outbreak to have been detected in that area, although sporadic cases have been seen in the District of Columbia hospitals since 1939.[1]

Presented herein are the case histories and laboratory findings during the primary phase of illness and follow-up x-ray, serologic, and skin test studies on 3 of the patients, made 4½ to 5 years after clinical recovery. The epidemiologic studies which led to the isolation of *Histoplasma capsulatum* from chicken compost to which the patients were exposed are described in a separate report.[2]

CASE REPORT

CASE 1.—A 33-year-old white man was admitted to the hospital May 3, 1952, because of fever of 10 days' duration. History revealed that his illness began with nausea, vomiting, diarrhea, headache, and generalized malaise. Three days after onset the gastrointestinal symptoms subsided but the headache and malaise persisted. He then noted chills and fever, accompanied by pain over the right lateral chest which was aggravated by deep breathing. Aureomycin for a period of 2 days was prescribed by his private physician but failed to relieve his symptoms. When Terramycin also failed, hospitalization was recommended.

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Physical examination disclosed a well-developed, well-nourished white man who appeared acutely ill. His skin was flushed and his temperature was 103° F. His pulse rate was 92 per minute. He had a blood pressure of 140 systolic and 90 diastolic. There was an exanthem over his forehead. His pharynx was injected slightly and a few scattered râles were heard throughout both lung fields. There was no cardiomegaly but a Grade 1 apical systolic murmur was heard with the pulmonic second sound being greater than the aortic second. A small tender lymph node, 0.5 by 0.5 cm. was felt in his left axilla but there was no generalized lymphadenopathy. Examination of the abdomen revealed no masses. The spleen and liver were not enlarged.

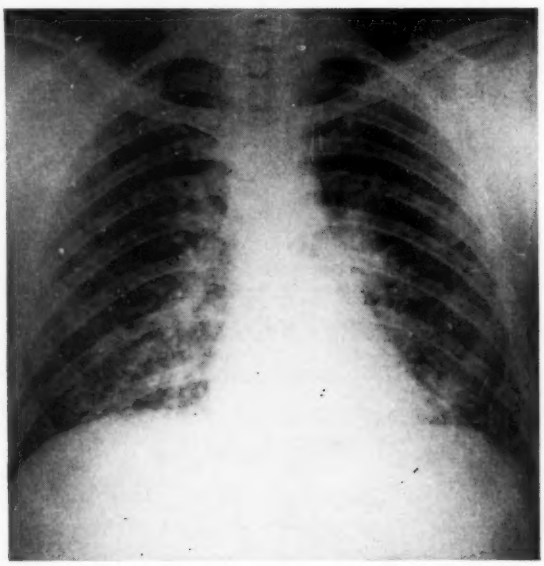

Fig. 1.—(Case 1.) Chest x-ray taken 12 days after onset of illness (May 4, 1952) reveals nodular and soft infiltrations throughout both lung fields in a miliary fashion.

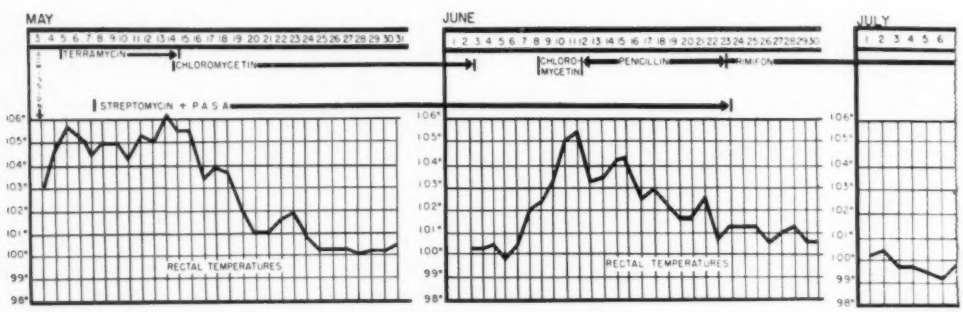

Fig. 2.—(Case 1.) Composite therapy and temperature (maximum for the day) course.

At the time of admission the hemogram revealed a white blood count of 5,900 with a differential of 50 neutrophils, 8 stabs, 45 lymphocytes, 3 monocytes, and 2 eosinophils. The corrected sedimentation rate was 32 mm. per hour, the hemoglobin 14 Gm., and the hematocrit 41 per cent. Urinalysis was negative. Agglutinations for the usual bacterial infections and complement fixation tests for syphilis, the psittacosis-lymphogranuloma venereum group of viruses, and Q fever were also negative. Bromsulphalein, thymol turbidity, and van den Bergh tests all were within normal limits; however, the cephalin flocculation test was 4 plus. Cultures

of sputum and aspirated bone marrow were negative for acid-fast bacilli. A chest x-ray taken the day after admission revealed nodular and soft infiltrations of a circumscribed nature that were diffusely scattered throughout both lung fields in a miliary fashion (Fig. 1).

The patient was isolated and treated supportively until specimens for further laboratory tests were obtained. Treatment with Terramycin, as indicated in the composite picture of the therapy schedule and temperature course shown in Fig. 2, was started on the second hospital day. On the fourth hospital day streptomycin and PAS were also instituted because of the miliary appearance of the lung lesions. However, when the patient's temperature remained elevated at 103 to 105° F. through the tenth hospital day, Terramycin was discontinued and

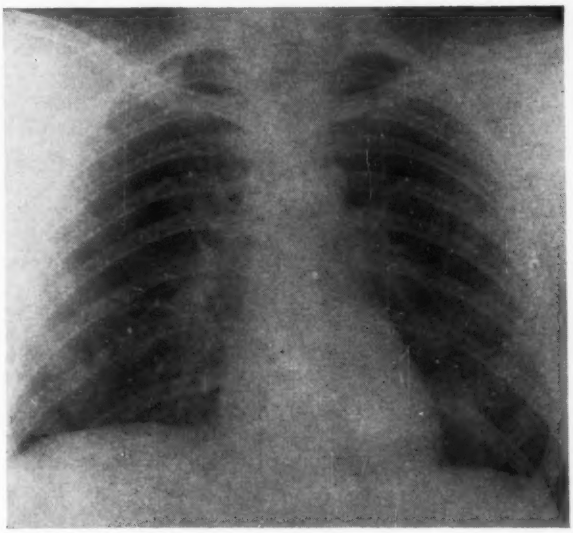

Fig. 3.—(Case 1.) Chest x-ray taken four months after onset of illness (Aug. 26, 1952) shows nodular densities ranging from the minute up to 3 mm. in diameter throughout both lung fields. There is no evidence of consolidation, cavitation, or pleural effusion.

TABLE I. SUMMARY OF SEROLOGIC DETERMINATIONS, CULTURE STUDIES, AND SKIN TESTS IN CASE I (FATHER)

TIME AFTER ONSET	COMPLEMENT FIXATION TITER: YEAST PHASE SOLUBLE ANTIGEN	COMPLEMENT FIXATION TITER: WHOLE YEAST ANTIGEN	COMPLEMENT FIXATION TITER: HISTOPLASMIN	COLLODION AGGLUTINATION TITER (HISTOPLASMIN)	ORGANISM ISOLATED FROM SPUTUM	SKIN TEST WITH HISTOPLASMIN
15 days	1:80	--	--	>1:40		
21 days					H. capsulatum	
28 days	1:80	--	--	1:40		
41 days	1:80	1:64	Negative	1:20		
71 days	1:40	1:32	Negative	1:40		
84 days	1:20	--	--	1:40		
111 days	1:20	1:8	Negative	1:40		
142 days	1:20	--	--	1:20	None	Positive
197 days	1:10	--	--	1:40		
4½ yr.	--	1:16	Negative	Negative		Positive*

*76 by 35 mm. erythema; 20 by 20 mm. induration.

Chloromycetin started. Following the second day of therapy with the latter drug, the temperature steadily declined to approximately 100° F. and continued at this level for the next 9 days. At that time, Chloromycetin was discontinued. Penicillin was then given for a period of 12 days, with a gradual lysis of temperature to 101 to 102° F. (rectal). On the fifty-first day of hospitalization the hydrazine derivative of isonicotinic acid, Rimifon (Hoffmann-La Roche, Inc.), in doses of 50 mg. three times daily, was also substituted for streptomycin and

PAS, and was continued until the time the patient was discharged approximately 7 weeks later. The temperature remained at 100.5 to 101° F. (rectal) during the first week of Rimifon treatment, then fell to normal limits of 99.6° F. Thereafter the patient was afebrile.

Additional laboratory studies revealed that the patient's serum which was first tested with mycotic antigens on the fifteenth day of illness fixed complement to a dilution of 1:80, and had a collodion agglutination titer of 1:40 with antigens of *Histoplasma capsulatum*. As shown in column 1, Table I, complement-fixing antibody was demonstrable at this level with the soluble yeast phase antigen through the fourth week of hospitalization, after which the titer decreased steadily to 1:10 during the following 4 months. However, the collodion agglutination test was still positive in a serum taken approximately 6 months after onset. Cultures of a sputum specimen collected 21 days after onset yielded *H. capsulatum*. On the other hand, direct cultures as well as those made from the spleens and livers of Swiss albino mice injected with a sputum specimen obtained 4½ months after onset were negative for this agent. The patient's skin test was positive to Histoplasmin when this test was made during the sixth month of disease. A tuberculin test with second strength P.P.D. was also positive while that with the first was negative. In serial chest x-rays from the time of admission through the fourth month there was a gradual diminution of the nodular densities throughout both lung fields, as revealed by the film taken on Aug. 26, 1952 (Fig. 3).

The diagnosis of histoplasmosis was supported by clinical symptomatology, isolation of *H. capsulatum* from sputum, significant antibody levels to histoplasma antigens in both complement fixation and collodion agglutination tests, a positive skin test reaction to Histoplasmin and roentgenologic evidence of pulmonary infiltration.

CASE 2.—About one week after the onset of illness in Case 1, that patient's 32-year-old wife, although never hospitalized, became ill. In addition to generalized malaise, her initial symptoms were headache, diaphoresis, and anorexia. These were accompanied by tightness in the chest and cough productive of purulent sputum, with occasional hemoptysis. There was no nausea, vomiting, or diarrhea. Her recovery was uneventful and apparently complete within one week, although no specific therapy was prescribed.

TABLE II. SUMMARY OF SEROLOGIC DETERMINATIONS, CULTURE STUDIES, AND SKIN TESTS IN CASE 2 (WIFE)

TIME AFTER ONSET	COMPLEMENT FIXATION TITER: YEAST PHASE SOLUBLE ANTIGEN	COMPLEMENT FIXATION TITER: WHOLE YEAST ANTIGEN	COMPLEMENT FIXATION TITER: HISTOPLASMIN	COLLODION AGGLUTINATION TITER (HISTOPLASMIN)	ORGANISM ISOLATED FROM SPUTUM	SKIN TEST WITH HISTOPLASMIN
10 days	1:5	1:64	Negative	1:40		
15 days					H. capsulatum	
24 days	Negative	AC*	AC	>1:40		
43 days	Negative	--	--	1:40		
105 days	Negative	--	--	1:10	None	
191 days	Negative	1:32	Negative	1:10		Positive
4½ yr.	--	1:8	Negative	Negative		Positive†

*AC = Anticomplementary.

†76 by 76 mm. erythema; 26 by 26 mm. induration.

As shown in Column 1, Table II, a serum obtained 10 days after onset fixed complement to a titer of only 1:5 with histoplasma soluble yeast phase antigen. All others in the serial series on this patient were completely negative for complement-fixing antibody. On the other hand, the collodion agglutination test was strongly positive in the initial specimen as well as in those collected on the twenty-fourth and forty-third days. Following this, the reactions with this test gradually became less intense. Cultures of a sputum specimen obtained 15 days after onset were positive for *H. capsulatum*. However, and as in Case 1, the organism was not recovered either in direct culture or from the livers and spleens of mice inoculated with a sputum specimen collected 4 months after onset. The Histoplasmin skin test, made 6 months after onset, was positive. A single chest x-ray obtained during the fourth week failed to reveal any evidence of pulmonary infiltration.

The diagnosis of histoplasmosis in this case was based on the isolation of *H. capsulatum* from sputum obtained early in the course of illness, the significant antibody levels demonstrated by the collodion agglutination test, and a positive skin test reaction to Histoplasmin.

CASE 3.—Also about one week after the onset of illness in Case 1, the patient's 14-year-old son became ill. Again, the symptoms were acute, with pain in the left chest, cough with production of purulent bloodstreaked sputum, anorexia, generalized malaise, and headache as the predominant ones. Temperature elevations of 103° F. were recorded during the first week. However, the patient made a rapid recovery and was able to return to school after an absence of approximately two weeks. Therapy included a total dosage of 2.5 Gm. Terramycin which was given every 4 hours in 250 mg. doses.

TABLE III. SUMMARY OF SEROLOGIC DETERMINATIONS, CULTURE STUDIES, AND SKIN TESTS IN CASE 3 (SON)

TIME AFTER ONSET	COMPLEMENT FIXATION TITER: YEAST PHASE SOLUBLE ANTIGEN	COMPLEMENT FIXATION TITER: WHOLE YEAST ANTIGEN	COMPLEMENT FIXATION TITER: HISTOPLASMIN	COLLODION AGGLUTINATION TITER (HISTOPLASMIN)	ORGANISM ISOLATED FROM SPUTUM	SKIN TEST WITH HISTOPLASMIN
10 days	1:40	1:64	Negative	1:20		
15 days					None	
24 days	1:40	1:256	1:32	1:40		
43 days	1:20	AC*	AC	1:10		
105 days	1:10	AC	AC	1:10		
191 days	Negative	1:128	1:256+	1:10		Positive
4½ yr.	--	1:16	1:8	Negative		Positive†

*AC = Anticomplementary.
†50 by 50 mm. erythema; 40 by 50 mm. induration.

As noted in Column 1, Table III, the complement fixation titer with histoplasma soluble yeast phase antigen was 1:40 in specimens taken on the tenth and twenty-fourth days after onset. This level decreased to 1:20 and 1:10 in serums collected at approximately 6 and 15 weeks, respectively. In a serum specimen taken 6 months after onset no complement-fixing antibody was demonstrable. Collodion agglutination tests in the tenth and twenty-fourth day serum specimens were also positive, whereas results with later specimens were equivocal. *H. capsulatum* was not isolated from the single sputum collected about 15 days after onset of symptoms. In the single chest x-ray obtained during the fourth week there was no evidence of pulmonary infiltration or hilar adenopathy. The patient, however, did have a positive reaction to a skin test with Histoplasmin when this was made approximately 6 months after onset of illness.

Although the diagnosis of histoplasmosis in this case was not supported by the recovery of *H. capsulatum*, the positive serologic and dermal sensitivity tests strongly indicated that the boy's infection was also due to this fungus, particularly since symptomatologically and in time of onset it closely simulated culturally verified Cases 1 and 2.

TABLE IV. SUMMARY OF SEROLOGIC DETERMINATIONS, CULTURE STUDIES, AND SKIN TESTS IN CASE 4 (SISTER-IN-LAW)

TIME AFTER ONSET	COMPLEMENT FIXATION TITER (YEAST PHASE SOLUBLE ANTIGEN)	COLLODION AGGLUTINATION TITER (HISTOPLASMIN)	ORGANISM ISOLATED FROM SPUTUM	SKIN TEST WITH HISTOPLASMIN
13 days	1:10	1:10		
18 days			None	
27 days	1:80	>1:40		
46 days	1:80	1:40		
108 days	1:20	1:20	None	
194 days	Negative	1:5		Positive
4½ yr.	--	--		--

CASE 4.—The fourth person involved in this series was the 27-year-old sister-in-law of Case 1 who resided with the family, and who was actively ill for only 2 or 3 days. Symptoms began about 2 days after the onset of illness in Case 1 and, although of shorter duration and less severe, they were similar to those experienced by the 3 other members of the family. She was away from her job for just one week. Since recovery was uneventful, no medication was prescribed.

In this patient, as illustrated in Column 1, Table IV, the antibody levels were very low in the earliest serum obtained. Fourteen days later, however, the complement fixation titer had increased to 1:80 and the collodion agglutination test had become markedly positive. These high antibody levels persisted for at least 3 more weeks and then, as in the other cases, gradually decreased. The patient was sensitive to Histoplasmin when tested 6 months after onset, in spite of the fact that cultures of two sputum specimens were negative for *H. capsulatum*. The single chest x-ray taken during the fourth week was negative.

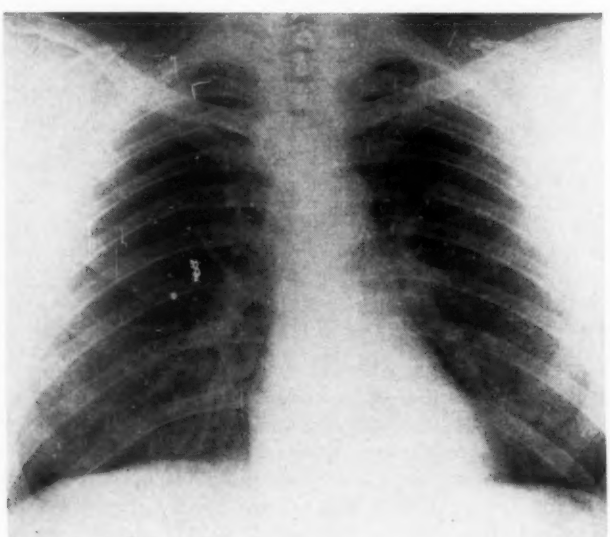

Fig. 4.—(Case 1.) Chest x-ray taken approximately 5 years after onset of illness (June 3, 1957) discloses no appreciable change from Fig. 3. The variable nodular densities are of comparable size, and no true calcification is seen.

The diagnosis of probable histoplasmosis in this case was based on a demonstrated increase in antibody levels to histoplasma antigens, a positive skin test to Histoplasmin, and on the similarity in the time of onset and clinical symptoms observed in Cases 1, 2, and 3.

The Follow-Up.—Additional data were obtained in 3 of the 4 cases 4½ to 5 years after the onset of their illnesses.

CASE 1.—At the time of the follow-up, the father appeared to be a vigorous, healthy man. His weight was 210 pounds, which represented a gain of 40 pounds over that maintained prior to his hospitalization with histoplasmosis. He resumed his occupation as butcher early in 1953 and has been employed continuously ever since. He has not had colds or further upper respiratory disturbances. As earlier noted, the serial chest x-rays (Fig. 1) from the time of his hospital admission in May, 1952, until 4 months thereafter showed a gradual and continuous diminution of the nodular densities throughout both lung fields (Fig. 3), but in the five years intervening there was no appreciable change. In a film taken in June, 1957, the nodular densities still varied from minute to 3 mm. in diameter and no true calcification was seen (Fig. 4). As shown in Column 2, Table I, this man maintained a C.F. antibody titer of 1:16 with whole cell histoplasma yeast phase antigen.

His skin test reaction to Histoplasmin also remained markedly positive. Complement fixation and collodion agglutination tests with the mycelial filtrate antigen, Histoplasmin, were negative.

CASE 2.—His wife also appeared to be well and healthy, and for the past 3 years has been employed as a stenographer in a large metropolitan bank. Approximately one year after the primary phase of her histoplasma infection she began to have low back pain. In the pyelograms made at that time there was a persistent filling defect in the region of the pelvis on the right due to some organic lesion. Whether or not this was an extrapulmonary focus of *H. capsulatum* was not determined. The symptoms eventually disappeared and there has been no recurrence. A chest x-ray taken in December, 1956, was negative, as was the earlier film made during the fourth week after onset of infection with *H. capsulatum* in 1952. Evidence of this patient's infection persists only in the residual C.F. antibody titer of 1:8 with the whole cell yeast phase antigen (Column 2, Table II) and her strongly positive skin test reaction to Histoplasmin (Table II).

CASE 3.—The son currently is an enlisted man in a branch of the United States Armed Forces. In the follow-up chest x-ray in December, 1956, there was a disseminated distribution of tiny calcified nodules, creating a "snow storm" pattern throughout both lung fields, regarded as very characteristic of healed miliary histoplasmosis (Fig. 5). This was in contrast to the negative findings during the fourth week of infection. A serum specimen also obtained in December, 1956, fixed complement to titers of 1:16 and 1:8 with the whole cell yeast phase antigen and Histoplasmin, respectively. His skin test reaction to Histoplasmin was strongly positive (Table III). A simultaneous skin test with P.P.D. was negative.

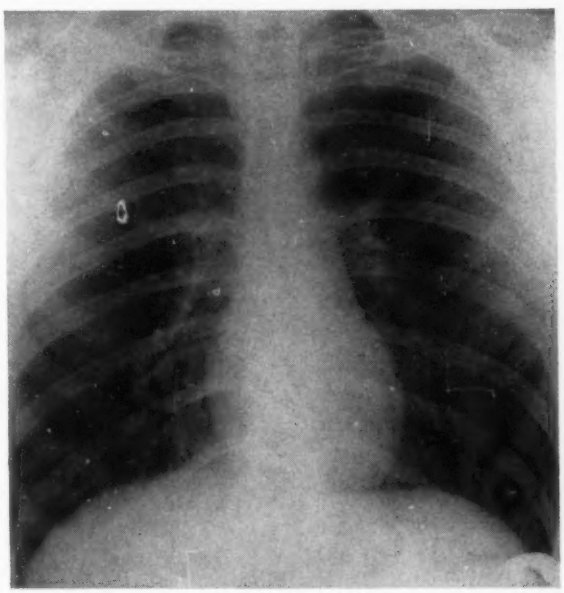

Fig. 5.—(Case 3.) Chest x-ray taken 4½ years after onset of illness (Dec. 22, 1956) shows a disseminated distribution of tiny calcified nodules throughout both lung fields.

CASE 4.—The sister-in-law apparently is also healthy and well. However, this patient moved to a distant state about two years after her histoplasmosis infection, and we were unable to gain her cooperation for follow-up studies.

METHODS

Serologic Tests.—The complement fixation (C.F.) and collodion agglutination (C.A.) tests employed were carried out as described in earlier reports.[3, 4] The antigen used for

the C.F. test in the primary phase of the study was the physiologic saline soluble component of yeast phase cells of *H. capsulatum* following disruption by grinding.[4] The whole cell yeast phase antigen and Histoplasmin,* a filtrate of the filamentous growth of the organism, were employed in follow-up C.F. tests.[5] Histoplasmin was the only antigen used in the C.A. test.

Skin Tests.—Follow-up skin tests were made with a 1:100 dilution of Histoplasmin.†

Culture Studies.—Early morning sputum samples were emulsified in 5 to 10 ml. physiologic saline solution containing 1,000 and 2,000 units of penicillin and streptomycin per milliliter, respectively. After an incubation period of one hour at 37° C., 0.5 ml. quantities were inoculated intraperitoneally into each of five 18 to 20 gram male albino mice and planted on brain heart infusion agar (Difco) containing 20 and 40 units of penicillin and streptomycin per milliliter, respectively, and one per cent dextrose. Plates of inoculated medium were incubated at room temperature for a period of 4 weeks. The mice were observed for 4 weeks, at which time all survivors were sacrificed and their livers and spleens cultured for *H. capsulatum.* The spleens and livers of mice dying after the seventh day were also cultured. Colonies appearing after the tenth day were transferred to Sabouraud's dextrose agar (Difco) and incubated at room temperature for final identification.

DISCUSSION

The clinical courses of the 4 persons involved in this family outbreak of primary, pulmonary histoplasmosis, the first to be reported from the District of Columbia area, were remarkably similar to those described in the epidemics report by Loosli and associates[6] and Furcolow and Grayston.[7] In 3 of the 4 persons, the symptomatology was relatively mild, transitory, and clinically indistinguishable from that in mild upper respiratory infections of viral or bacterial etiology. In fact, had it not been for the grave illness in Case 1 it is doubtful that the milder illnesses would have been ascribed to any specific agent, much less to the fungus, *H. capsulatum.* Despite the variation in the degree of severity, however, all made spontaneous and apparently complete recoveries.

In order to establish that an outbreak of pneumonitic illnesses is due to *H. capsulatum,* it is sometimes necessary to rely on indirect criteria as outlined in the studies of epidemics by Grayston and Furcolow.[8] In the present one, however, the rapid demonstration of agglutinins and complement-fixing antibodies with histoplasma antigens in early sera from each of the patients led to immediate cultural studies. The latter yielded *H. capsulatum* not only from two of the persons involved, but ultimately also from the contaminated chicken compost to which all 4 had been recently exposed.[2] Skin tests with Histoplasmin were also positive.

This series of cases also provided additional evidence that therapy with antibacterial antibiotics has little if any beneficial effect on the course of primary, pulmonary histoplasmosis. Two of the four cases were untreated and a third received a total dosage of 2.5 Gm. Terramycin. The fourth case was treated with a variety of drugs, including Terramycin, Chloromycetin, penicillin, Rimifon, streptomycin, and PAS. In spite of this, this patient's stormy, prolonged course was essentially similar to that in other reported cases of equal severity who recovered without benefit of specific therapy. Loosli[9] and Silverman and his associates[10] describe severe infections in which recovery occurred without the use of antibacterial antibiotics or drugs of any kind. Moreover, there is some

*Lot H-15: Parke, Davis & Company, Detroit, Mich.
†Lot 029386-F-029975-F: Parke, Davis & Company, Detroit, Mich.

evidence that streptomycin, at least, actually enhances rather than inhibits the growth of mycotic agents.[11]

Of special interest were the follow-up findings relating to pulmonary involvement. Extensive infiltration (Fig. 1) was observed during the initial phase of the study only in the person with the most severe illness. Although there was considerable clearing during the first 4 months (Fig. 3), since that time there has been no essential change either in the areas of nodular densities or size of the individual lesions. Nor is there evidence of calcification (Fig. 4). In contrast, the follow-up film in Case 3 revealed a typical "snow storm" pattern of calcification (Fig. 5) although the single film obtained on this patient during the fourth week after onset was negative. The initial and follow-up films in Case 2 were both clear. Whether infiltrates might have been present at some time prior to or later than the fourth week in either of these two milder cases, of course, cannot be ascertained. From the still negative skin test reaction to P.P.D. and the extensive calcification in Case 3, however, it is not unlikely that at one time there was considerable infiltration in this patient. For this reason, serial chest x-rays appear to be as important as serial serologic and repeated cultural studies in following the early suspected and verified cases of histoplasmosis. The reported absence of pulmonary infiltration during the primary episode even in relatively mild infections perhaps should be viewed with caution unless there are serial x-rays at rather close intervals to substantiate this point.

Serologic tests, as noted above, were used to good advantage in the detection and study of these cases. Cultural studies were initiated only after it was known that serologic tests were positive. The importance of obtaining specimens (sputum, gastrics and blood) for culture during an acute phase of this infection has perhaps received too little emphasis. Experience in this laboratory reveals that it is during the early stage of infection that the organism is most readily isolated. In this regard the collodion agglutination test is especially useful. This test which can be performed in any hospital laboratory within a period of 3 hours directs attention to the possibility of primary pulmonary histoplasmosis during a phase of active infection when cultural and additional serologic studies yield the most definitive and valuable information. Agglutinins, moreover, are usually demonstrable by the time symptoms begin to occur even in cases which are not sufficiently severe for the development of detectable complement-fixing antibodies.[12, 13]

As illustrated in Tables I, II, and III the results of complement fixation tests vary with the type of antigen, duration and type of disease, and other as yet undetermined factors. For example, the reactions with whole yeast phase antigen in sera from Case 1 were essentially the same as those with the soluble antigen, whereas those in sera from Cases 2 and 3 were considerably higher with the whole cell preparation. Later sera from Case 3 also reacted to a titer exceeding 1:256 with Histoplasmin, in contrast to negative findings with this antigen in Cases 1 and 2. The importance of employing different types of antigenic preparations is discussed in an earlier report.[5] There is at the present time little doubt that the whole cell antigen is superior to the soluble yeast phase component; that the reactions obtained with the whole yeast cell antigen also sometimes differ markedly from those obtained with Histoplasmin; and that until these differences are resolved both types of antigens should be employed in complement fixation

tests for histoplasmosis. However, it is not unusual for a low residual C.F. antibody titer or a marked skin test sensitivity to persist for an, as yet, undetermined number of years following a primary infection, as illustrated by the findings in these cases. Their interpretation awaits the close study and follow-up of an increasing number of cases from the time of onset of infection as exemplified by the investigations of Loosli and his associates.[6]

SUMMARY

An epidemic of primary, pulmonary histoplasmosis in a family of 4 persons living near Washington, D. C., is described. The father was critically ill for a period of 3 months, whereas the infections in his wife, son, and sister-in-law were only mild to moderately severe. All recovered. *Histoplasma capsulatum* was isolated from the sputums of the husband and wife. Elevated titers of agglutinins and C.F. antibodies were demonstrated in the sera of all 4 persons and all were skin test positive to Histoplasmin. Although the father was treated with a variety of antibiotic agents, none of the agents appeared to alter his stormy course. Follow-up chest x-rays and skin and serologic tests were obtained 4½ to 5 years later on 3 of the patients. In the son's film there was a typical "snow storm" pattern of calcification (Fig. 5) whereas in the father's there was no calcification and no appreciable change in nodular densities compared with 5 years before (Figs. 3 and 4). The film on the wife was negative. Residual C.F. titers of 1:8 and 1:16 and strongly positive skin test reactions to Histoplasmin persisted in all 3 persons. Studies leading to the isolation of *H. capsulatum* from contaminated chicken compost to which 4 persons were exposed approximately two weeks prior to onset of their infections are described in a separate report.[2]

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A FAMILY OUTBREAK OF HISTOPLASMOSIS

II. Epidemiologic Studies

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THIS report is concerned with the epidemiologic aspects of an outbreak of histoplasmosis involving all the members of a family.[1] These individuals were residents of Glenmont, a suburban housing development situated within 5 miles of the city limits of Washington, D. C., in Montgomery County, Maryland. The epidemic, although similar in many respects to others earlier reported,[2-4] was of special interest because it occurred in a geographic area of "low" endemicity and involved persons who lived in a suburban rather than rural area. These findings are conspicuous only in that they invalidate certain not altogether warranted impressions that have arisen during the last 10 years concerning the acquirement of this infection.

The purpose of this report, therefore, is not only to describe epidemiologic studies in an additional outbreak of histoplasmosis, but also to emphasize that: (1) the geographic distribution of *Histoplasma capsulatum* has not been fully outlined; (2) maps of endemicity in many instances reflect the extent to which the disease has been investigated in particular localities rather than actual prevalence of the infection; thus, in areas of purportedly low endemicity the real prevalence of the infection is often unknown; (3) exposure to the etiologic agent in nature is not necessarily associated with dusty environments or uncommon activities of prolonged duration; and (4) infections are not limited to rural residents or other isolated groups.

The Family.—This family comprised 4 persons: the husband, his wife, their 14-year-old son, and a sister-in-law. The father was employed as a butcher in an Army commissary and the wife and sister-in-law as stenographer and clerk-typist, respectively, by different commercial firms. The son attended high school. Week ends were used for gardening, housekeeping, marketing, and similar duties. The family occasionally took automobile rides on Sunday, but on these occasions did not visit farms or take walks over country terrain. There had been no vacations, either separately or together, for at least 8 months. The father, at the time of onset of illness, was terminating a convalescent period following surgery for hernia but other members of the family had been healthy and well prior to onset of their respiratory infections.

The Outbreak.—In April, 1952, each of these persons experienced an episode of primary pulmonary histoplasmosis.[1] The diagnosis was unequivocally established in the husband and wife by the isolation of *H. capsulatum* from their sputums, and presumptively in the son and sister-in-law by a demonstrated increase in histoplasma antibody as well as similarity in symptomatology and time of onset to the two verified cases. The husband's episode was one of extreme gravity and required a 3-month period of hospitalization for recovery. The son's, wife's, and sister-in-law's illnesses, requiring only 1 to 2 weeks of bed rest at home, were considerably less severe. An investigation to find the point source in nature from which these infections were acquired was initiated at once. All available evidence indicated that this source existed in the District of Columbia area.

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The Environment.—The Glenmont housing development consisted of an area approximately two miles square. Modest clapboard houses of relatively contemporary design occupied lots approximately 60 feet wide and 100 feet deep, and were fronted by concrete sidewalks and paved streets. At the time of the outbreak, there was no evidence of construction involving the use of bulldozers or other dust-raising equipment within a radius of one-half mile. The narrow front lawns were sodded and well kept.

The house in which this family had lived for the past 5 years was essentially the same as many others in the immediate and surrounding blocks. Like these, it was situated near the front of the lot to allow space for small vegetable or flower gardens as well as additional lawn in the rear. Because of the season many of the gardens had recently been plowed and planted. This family's garden adjoined that of the neighbor's to the rear and those of the neighbors' on both sides. An occasional lot was partitioned by a low wire fence. More often, there was no demarcation, and the individual properties were continuous, permitting free access from one to the other. The few trees appeared to be young and vigorous.

FIELD STUDIES

Early discussion with the father revealed that while he could recall no particular activity in which the family had participated collectively, he and his son had cleaned the basement of their home approximately 10 days before the onset of their illnesses. This had proved to be an especially dusty and time-consuming task as the son, who was an amateur naturalist, used it as a workshop. There he prepared and preserved for display or kept as pets for indefinite periods birds, their nests and eggs, and small rodents which he collected during his frequent trips to a little wood about one mile south of the development. Neither the wife nor the sister-in-law was at home when the dust was at its highest concentration, but both made brief excursions to the basement later in the day. Moreover, sufficient dust had escaped to the upper story to necessitate a thorough cleaning of the house. It was also recalled that 3 baby squirrels were brought into the house about one month before the onset of these illnesses. The animals died after several days, allegedly of malnutrition, but actually of unknown causes. Other possible contributors to the outbreak were the several birds' nests, wings from a sparrow hawk, the large eggshell collection on display in the boy's room, and the dog and three cats owned by the family. The technique for emptying eggshells was especially suspect since it involved bathroom towels in common use. Finally, it was learned that game birds, following removal of their wings for preservation, were often eaten by all persons concerned in the outbreak.

In spite of this array of possibilities, attention was focused on the basement as the most likely source. This was a large, noncompartmented room with a concrete floor and walls of the cinder block type. It was accessible only by a stairway leading from the kitchen. Relatively dry and well lighted, it contained the usual permanently installed laundry tubs and furnace, but no storage bins or chests. Because of the recent cleaning, little remained to be collected as specimens for mycologic study. Owl pellets that were overgrown with a species of nonpigmented fungus were found on the floor in one corner. These, a 50 by 70 cm. mat of fungus about two feet distant from the pellets, and a green mold covering one of the glass eyes on a stuffed buffalo head were the only specimens returned to the laboratory for study.

A second trip to the home was made three days later. On this occasion specimens from the surrounding environs as well as additional ones from the

basement were collected. These included: (1) washings sponged from each of the basement walls; (2) soil samples from 4 different areas in the backyard and garden, 3 were selected because they were the possible but completely forgotten burial sites for the baby squirrels and the fourth because it comprised the floor of a wire chicken pen used for caging captured birds, and only rarely for housing chickens prior to slaughter; (3) nesting materials from the wooden box in which the baby squirrels died; (4) a walnut shell filled with mycelium also found in this box; (5) a pigeon's nest from a high rafter in a deserted barn approximately one mile distant from the house, from which the son often collected specimens of eggs and birds; and (6) the owl's nest, by this time abandoned, from which the pellets found earlier on the basement floor originated.

As shown in Table I, *H. capsulatum* was not recovered from any of these specimens. Negative results were also obtained by two other investigators to whom portions of many of these materials were sent.* It was of interest, however, that *Cryptococcus neoformans* was isolated from the pigeon's nest by each of the persons who studied it.[5, 6]

TABLE I. SUMMARY OF ISOLATION STUDIES FOR HISTOPLASMA CAPSULATUM ON SPECIMENS FROM SUSPECTED EXPOSURE SOURCES (GLENMONT OUTBREAK, MONTGOMERY COUNTY, MD.)

NO.	SPECIMENS	MYCOTIC PATHOGEN ISOLATED	
		CULTURE	MOUSE INOCULATION
	Collected May, 1952		
1	Owl pellets	None	None
2	Mycelial mat (50 × 70 cm.)	None	None
3	Green mold on glass eye of buffalo head	None	None
4	Basement wall washings	None	None
5	Soil sample No. 1	None	None
6	Soil sample No. 2	None	None
7	Soil sample No. 3	None	None
8	Soil sample No. 4 from chicken coop	None	None
9	Nesting material in squirrel box	None	None
10	Mycelium filled nutshell	None	None
11	Owl's nest	None	None
12	Pigeon's nest	*C. neoformans*	*C. neoformans*
	Collected December, 1956		
13-21	Soil samples from garden	Not done	None
22	Soil sample from chicken house floor	Not done	*H. capsulatum*
23	Soil sample from chicken house floor	Not done	*H. capsulatum*
24	Soil sample from chicken house floor	Not done	*H. capsulatum*
25-27	Soil sample from chicken house floor	Not done	None
28-30	Soil with crumbling wood from slatted platform in chicken house	Not done	None

In spite of these negative findings, it was still believed that the basement was the only common source to which all of these persons could have been exposed. Our failure to recover *H. capsulatum* from any of the above specimens was attributed to the fact that the offending focus was removed from the basement during the cleaning operation before our first trip to the house. For this reason, further studies did not appear to be warranted.

In December, 1956, approximately 4½ years after the onset of these infections, follow-up x-rays and skin and serologic tests on 3 of the 4 persons were

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obtained.[1] When the father returned for his follow-up test, he was accompanied by his ailing mother-in-law in order that skin and serologic tests might also be made on her. When it was disclosed that the mother-in-law resided on a small farm near Waycross, Georgia, and that she tended a small flock of chickens, considerable emphasis was placed on whether or not she also cleaned the hen house. On being advised of the close association of *H. capsulatum* spores and soils enriched with chicken excreta,[7] the father instantly recalled that he had collected, hauled, and spread chicken manure on his own garden the day before cleaning the basement in his home.

Armed with this heretofore undisclosed information, a third trip was made to collect soil samples. Soils were taken from nine areas in the garden, which during the intervening years had been repeatedly plowed, fertilized, and planted. Since the chicken house from which the manure was initially obtained was not more than one mile east of this suburban housing development, specimens of soil were also collected from it. This small dilapidated structure, no longer suitable for housing chickens because of a leaky roof and missing planks on the sides, was empty except for a barrel and a few chunks of fire wood. Six samples of soil were collected from various areas of the moist dirt floor and 3 additional ones from a slightly elevated platform on which the crumbling fire wood was stacked.

As shown in Table I, *H. capsulatum* was readily recovered from mice inoculated with 3 of the 6 soil samples collected from the dirt floor of the chicken house. Specimens from the slatted platform as well as the garden areas were negative. It is of interest to note that the organism was isolated in one instance as early as two weeks after injection, indicating that even though 4½ years had elapsed the spores were still present in high concentration in this particular sample.

LABORATORY STUDIES

1. *Materials from Sources in Nature.—*

Soil samples: For those samples collected during the initial phase of this study three different preparation techniques were employed in order to utilize each of the various modifications described by Emmons,[8] Zeidberg and Ajello,[9] and Larsh and associates[10] for isolating *H. capsulatum* from soil. Only the method described by Larsh and associates[10] was used on the later samples. These suspensions were used only in mice.

Bird and animal nests: Approximately 2 grams of twigs, leaves, feathers, and soil were selected from different parts of the nests, broken into minute fragments, placed in a 100 ml. cylinder containing 30 ml. of physiologic saline, and shaken vigorously. After 10 minutes, the supernatant fluid was siphoned from the sedimented larger particles and centrifuged at 5,000 r.p.m. for 5 minutes. The sediment from the latter, resuspended in 10 ml. of saline, comprised the inoculating suspension for mice. No direct platings on culture medium were made.

Basement wall washings: Areas, approximately 4 feet square, on each of the 4 basement walls were swabbed with sterile sponges moistened and later rinsed in 1,000 ml. sterile, distilled water. Debris from the latter was collected on millipore filters (Type HA) and resuspended in 10 ml. saline.[11] Media as well as mice were inoculated with these suspensions.

Miscellaneous specimens: Other specimens such as owl pellets, the several specimens of fungi, nutshells, etc., were each suspended in 30 ml. of physiologic saline, shaken vigorously, and allowed to stand in 100 ml. cylinders for one hour. Aliquots of 5 ml. were removed separately from the surface, middle, and supernate-sediment interface of each suspension for injection into mice and inoculation of selective media.

2. *Media.*—Brain heart infusion agar (Difco), to which glucose, penicillin, and streptomycin were added in final concentrations of 2 per cent and 20 and 40 units per milliliter of medium, respectively, was the sole substrate for culturing livers and spleens of inoculated mice. Sabouraud's agar containing cycloheximide was used for the direct culture of many of the specimens collected from nature.[12]

3. *Animal Inoculations.*—Male albino mice in groups of 10 and weighing 18 to 20 grams were used. They were inoculated intraperitoneally with 0.5 or 1.0 ml. volume of the prepared specimens, depending upon the quantity available. All were treated daily for 7 days following inoculation with 1,000 units of penicillin and 2,000 units of streptomycin. The animals were observed for a period of 4 weeks, at which time half of the survivors were sacrificed and their livers and spleens cultured for *H. capsulatum*. The remaining mice were sacrificed and their livers and spleens cultured at the end of 8 weeks. The spleens and livers of mice which died after the seventh day were also cultured.

Except for those soil samples collected during the follow-up phase of the study, this series of procedures was repeated 3 times in this laboratory on separate aliquots of each of the specimens collected from nature. In one of these trials, 5 per cent hog gastric mucin was substituted for physiologic saline as the suspending menstruum for materials injected into mice.[13, 14]

DISCUSSION

Although forests, chickens, birds, rodents, and even domestic pets were involved in this epidemiologic investigation—and all are implicated as possible contributors to the distribution of *H. capsulatum* in nature[3, 5, 15-20]—the agent finally was recovered from the source most commonly associated with primary, pulmonary infections of histoplasmosis; soils enriched with the excreta of chickens or other fowl.[5-7]

It was nevertheless of interest to learn that in the contracting of these infections the time of exposure was so brief and the source so usual that neither was recalled until specific questions were asked. In this instance, the initial period of exposure could not have exceeded a period of one hour and, unlike more dramatic undertakings such as speleologic expeditions and the cleaning of silos, water towers, or even dusty basements,[2-4] it was associated with a very commonplace task. The father, also accompanied by his son on this occasion, had driven to the nearby farm, hurriedly scooped the soil into small kegs, transported it home in the trunk of his car and deposited it on the garden. Yet the ready recovery of *H. capsulatum* from 3 of the 6 soil samples collected several years later from this original source leaves little doubt that it was in the execution of this incidental chore that the near fatal and moderately severe illnesses were incurred. For the wife and sister-in-law, the means of exposure was even less impressive. Neither was directly involved in this task. They merely pulled weeds in the garden within the next few days.

Nor can it be assumed that these were the only cases among the many persons having free access to this garden. The initial studies included a serum from a 12-year-old boy who lived across the street and whose upper respiratory infection coincided in time of onset and duration with that of the son's. A titer of 1:128 in serologic tests left little doubt that his infection was one of histoplasmosis acquired in a manner similar to the others because of his frequent visits to this home. Therefore, in our excessive concern over the dusty basement we had failed to investigate the more serious illness in the neighbor who lived to the rear, despite the fact that her bout with bronchopneumonia was

sufficiently severe to require 3 weeks of hospitalization. She, indeed, had not visited this family, but her garden directly adjoined their contaminated plot. In retrospect, it is thus not unlikely that her infection and possibly some of the milder ones that beset residents of this community was due to exposure to this at least temporary focus of *H. capsulatum*.

This series of cases comprises only a minor part of the evidence that the District of Columbia area is one of high endemicity for histoplasmosis, contrary to the relatively low prevalence assigned to it on distribution maps.[3] *Histoplasma capsulatum* is isolated repeatedly from the soils of nearby communities by Emmons as well as ourselves.[5, 21,23] Moreover, as disclosed by our own serologic studies carried out cooperatively with physicians in this area, there were 33 cases with serologic titers of 1:16 or above with histoplasma antigens in residents of the District of Columbia and adjoining states within the past year. Patients in military installations in these states account for 128 additional cases with a presumptive serologic diagnosis of histoplasmosis within the same period.[23] These figures included only those persons with illnesses of sufficient severity to require hospitalization.

In view of the above, it is therefore pertinent to point out that distribution maps might not be entirely accurate for other regions assigned a low prevalence, but where, as in the District of Columbia, the incidence is actually unknown. In this regard, it is not generally recognized that skin test surveys with Histoplasmin, the principal method for determining the distribution of this agent, have not been carried out with the same intensity in all areas of the United States. In addition, it has not been sufficiently emphasized that even in areas known to be endemic the organism's distribution is spotty rather than continuous.[7, 9, 24] In a recent and more complete skin test survey in Pennsylvania, for example, Aronson and Taylor found a high rate of Histoplasmin sensitivity in the counties comprising the southeastern part of the state, in marked contrast to the low incidence ascribed to the state as a whole on the basis of previous, sporadic studies.[25] It was thus not surprising when the organism was subsequently isolated from a soil sample collected in Chester County, Pennsylvania, and from the native resident whose pulmonary infection was traced to exposure to that source.[23] Nor is it unlikely that there are other primary infections in that area which continue to be overlooked or attributed to other more usual causes. A similar situation undoubtedly exists in certain areas of New York where primary, pulmonary cases in residents exposed to a local focus of the organism were recently described.[3, 19]

The finding of an increased number of cases in any area is generally due to a greater awareness of the disease and improved diagnostic methods. Nevertheless, there are relatively new situations in which heterogenous populations rather than isolated groups might be expected to be exposed more frequently to this agent. Primary among these are the new suburban communities in various stages of development throughout the nation. These vast communities usually not only occupy territory that was farm land a short while before but are adjacent to others still used for this purpose. In many of these communities, gardening is a common pursuit. The readily accessible compost, as in this

series of cases, is transported through densely populated areas in cars or trucks and, if contaminated, at least temporary foci are established in the vehicles, the routes they travel and the areas on which the compost is eventually spread. The automobile which figured in this outbreak, for example, was traded for a new one within the week. What of the new purchasers who were exposed to the spore-laden trunk? And would such exposure constitute an unusual activity to which later infections might be traced?

Finally, there appears at the present time to be sufficient evidence to indict the chicken house, especially the old, unused one, as a prominent reservoir of this fungus, and possibly to use it as one of the indices as to whether histoplasmosis can be expected to occur in a community or not. The close association of *H. capsulatum* and soils enriched with excreta of chickens or other fowl was first pointed out by Zeidberg and Ajello in studies carried out in Tennessee[7]; a curious predilection that has been amply verified by the extensive studies of Emmons as well as more limited ones by others in various eastern states.[3, 5-7, 9, 19, 21-23, 26] Our own experiences emphasize that it is the soil from the old and often abandoned coop, or areas on which such buildings have recently stood, that is particularly suspect. These rich soils, in addition to having been undisturbed, usually are exposed to moisture but not to sun, and thereby are ideal environments for the luxuriant growth of this organism.[21, 27, 28] In the present study, it is to be noted that the organism was readily recovered from the source which met these requirements, but not from the garden which was continuously exposed to radiation. The eastern seaboard states are densely populated with old towns and villages and the newer type of community described above. All are heavily endowed with old chicken houses, many of which are no longer used. It is perhaps time to question whether they are merely eyesores and as innocuous as they seem, and to regard them as potential reservoirs of *H. capsulatum* regardless of their geographic area.

SUMMARY

Four cases of primary, pulmonary histoplasmosis occurred in a family living in a suburban housing development near Washington, D. C. Locating the exposure source of *H. capsulatum* was complicated by the family's close association with a variety of possible foci. These included decaying wood, birds, their nests and eggs, rodents, domestic pets, and, a singular but unrelated incident involving a high concentration of dust. During a follow-up study 4½ years after the primary infections, *H. capsulatum* was isolated from the dirt floor of an old chicken coop on a nearby farm. The father and son had collected chicken compost from this coop to fertilize the family garden about 10 days prior to onset of their infections. The mother and sister-in-law worked in this garden during the next few days.

Because of the increasing evidence that the District of Columbia area is one of high rather than low endemicity for this fungus, some of the limitations of the currently available map of distribution are pointed out. The association of exposure to this organism with commonplace tasks, a possibly increasing

number of infections in heterogenous populations of suburban communities, and the prominence of the old chicken coop as a reservoir of *H. capsulatum* are also discussed.

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GENERALIZED EXCESSIVE OOZING IN PATIENTS UNDERGOING MAJOR SURGERY AND RECEIVING MULTIPLE BLOOD TRANSFUSIONS

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GENERALIZED oozing from small blood vessels in the operative field may occur during surgery. Usually it is short-lived and minor in degree. On other occasions it may be excessive and prolonged or intractable. The cause is not well understood. Massive blood replacement,[1-4] a demonstrable circulating fibrinolytic enzyme,[5-11] hypo or afibrinogenemia, and platelet deficiencies have all been suggested as the cause.[10-13]

A number of factors concerned with blood clotting and fibrinolysis have been measured before, during, and after surgery. An attempt has been made to correlate changes in these factors both with the occurrence of excessive oozing during surgery and with the number of transfusions administered.

Table I. Incidence of Oozing and Fibrinolysis in Relation to Site of Operation

Site of Surgery	Total Number Patients	Number Showing Oozing	Number Showing	
			Euglobulin Activity	Whole Clot Lysis
Head and neck	16	1	6	2
Thoracic	7	1	1	0
Abdomen and G. U.	48	9	13	5
Hepatic lobectomy	7	4	4	3
Total	78	15	24	10

MATERIAL AND METHODS

Most of the patients studied were undergoing extensive surgery for malignant neoplasms (Table 1). Venous blood samples were drawn on the day before the operation, on

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TABLE II. PERTINENT DATA AND MAXIMUM CHANGES OBSERVED IN PATIENTS WITH EXCESSIVE SURGICAL BLEEDING

		AGE	SEX	SITE OF SURGERY	NO. UNITS BLOOD	EUGLOBULIN (HR.)	WHOLE BLOOD (HR.)	PROTHROMBIN TIME (SEC.)	PROTHROMBIN CONTENT (%)	FACTOR V (%)	FACTOR VII (%)	FIBRINOGEN (MG. %)	PROTHOMBIN CONS. (%)	PLATELET COUNT 10^3/C.MM.	HYPOTENSION
	Series I														
1.	S. B.	60	M.	Laryngectomy and neck dissection	6	$>8<16$*	—	17.2	90	78	—	160	>90	205	No
2.	C. D.	33	F.	Anterior pelvic exenteration	7	1	3	17.1	52	54	—	160	>90	112	No
3.	O. R.	41	F.	Cholangio-enterostomy	4	10	10	—	70	77	—	58	—	268	No
4.	P. S.	62	F.	Hysterectomy and node diss.	3	½	—	—	—	—	—	—	—	—	No
5.	C. W.	73	F.	Total pelvic exenteration	6	4	16	17.0	72	56	55	480	>90	190	No
6.	C. S.	55	M.	Right hepatic lobectomy	15	1	2	18.9	69	42	75	<100	>90	183	No
7.	W. W.	48	M.	Right hepatic lobectomy	29	4	12	18.4	46	73	62	39	90	117	2 hr.
	Series II														
1.	C. H.	46	F.	Cholecystectomy	0	$>8<20$*	>24	14.9	60	71	100	440	32	—	No
2.	A. K.	68	M.	Pneumonectomy	4	⅔†	>24†	19.0	86	42	59	345	>90	250	No
3.	J. T.	48	M.	Laparotomy inoperable liver hemangioma	3	5‡	>24‡	16.0	88	65	52	224	75	225	No
4.	T. A.	52	F.	Exenteration nephrectomy	11	>24	>24	15.2	70	61	100	375	78	112	No
5.	A. M.	57	F.	Hemipelvectomy	7	>24	>24	16.3	80	56	61	295	>90	195	No
6.	M. R.	29	F.	Exenteration	8	>24	>24	15.3	59	60	78	375	90	295	No
7.	E. J.	60	F.	Right hepatic lobectomy; posterior gastrostomy	14	1	$>1<5½$	17.5	70	40	59	340	>90	150	1 hr.
8.	M. S.	49	F.	Right hepatic lobectomy	7	$>8<17$*	>24	19.8	62	—	52	422	—	240	No

*Since lysis may not have occurred within 10 hours, these patients are not considered to have had significant euglobulin fibrinolytic activity.

†Sample taken 40 minutes after closure. The previous sample was taken 2½ hours before the end of a 6 hour operation and did not show fibrinolytic activity. Oozing occurred 30 minutes before closure.

‡Sample taken 20 minutes after closure. Oozing occurred during closure. A sample taken 30 minutes before oozing was noted had no fibrinolytic activity.

the day of operation before and after the induction of anesthesia (thiopental, nitrous oxide, and ether), and at intervals during and after surgery. Fibrinogen and fibrinolysin determinations were done on all samples, and other studies outlined below were done in most instances.

The following determinations were made:

1. Fibrinolytic Activity of Whole Blood: The time required for complete lysis of a whole blood clot was determined at 37° C.

2. Fibrinolytic Activity–Euglobulin Fraction: A previously reported modification[14] of the method of Milstone[15] was used. In the early cases 50 units of thrombin were used but later this was changed to one unit of thrombin.*

3. Fibrinogen: Quick's modification of the method of Cullen and Van Slyke.[16]

4. Platelet Count: This was performed on capillary blood.[17] Normal values averaged about 250,000 per c.mm.

5. Prothrombin Time: Quick's method.[16] Normal values were less than 14 seconds.

6, 7, and 8. Prothrombin, Factor V, and Factor VII. Modifications of Owren's one stage assays were used.[18] Normal values were above 70 per cent.

9. Prothrombin Consumption: Prothrombin determinations were carried out on serum from blood oxalated after one hour at 37° C.[18] Normally, over 90 per cent of the plasma prothrombin was consumed.

10. Citrate Concentration: This was determined by the Natelson method[19] modified by Elliot.[20]

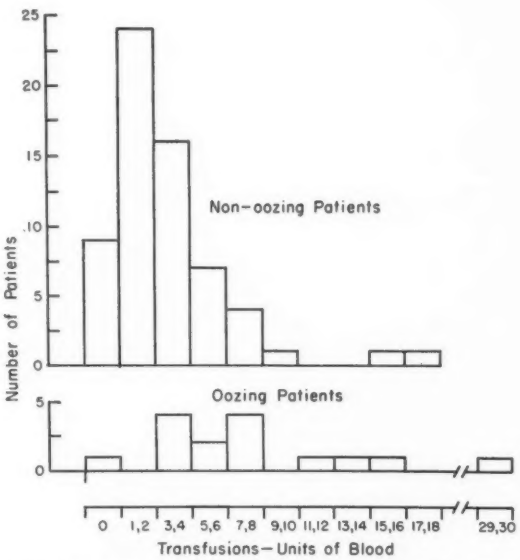

Fig. 1.—Number of transfusions given to nonoozing and oozing patients.

RESULTS

Excessive generalized bleeding or oozing occurred in 15 of the 78 patients studied. It was observed during many types of operations but most frequently during hepatic lobectomy. (Tables I and II).

Massive Blood Replacement and Changes in Platelet Count.—Four of the 15 oozing patients received over 10 units† of blood while only two of the 63

*Bovine Thrombin: Parke, Davis & Company, Detroit, Mich.

†One unit is 600 ml. A.C.D. bank blood, which contained 480 ml. or 1 pint of blood and 120 ml. of A.C.D. The average storage age of 295 units of bank blood given to 21 patients was 7.8 days.

patients who did not ooze received this much blood (Fig. 1). A significant relationship between oozing and massive transfusion (Chi square = 6.36; P 0.02 = 5.41; P 0.01 = 6.64) is indicated, but it is impossible to prove that the multiple transfusions were not the effect of rather than the cause for the excessive bleeding.

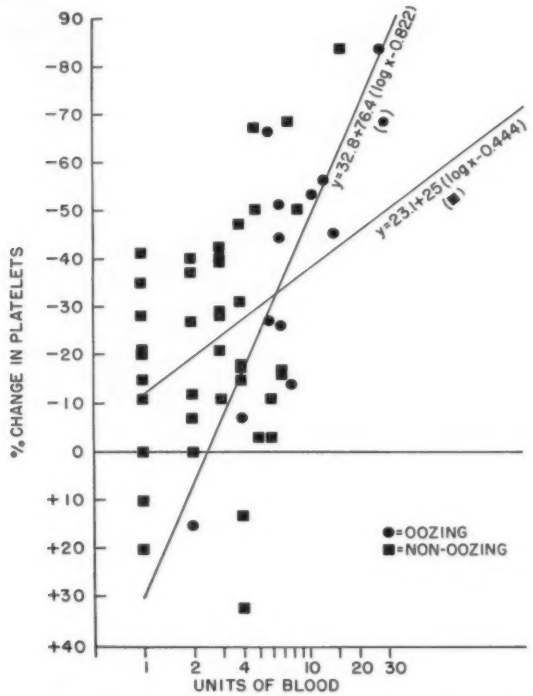

Fig. 2.—Relation between number of transfusions and per cent change in platelet count in oozing and nonoozing patients.

The platelet count after transfusions was compared with the preoperative count, and the per cent change plotted against the number of transfusions for 13 patients who oozed and 40 patients who did not ooze (Fig. 2). The average control platelet count was 329,000/c.mm. and was over 400,000/c.mm. in 25 per cent of the patients. The platelet count fell sharply after multiple transfusions. However, only two patients, neither of whom oozed, had platelet counts below 100,000/c.mm. after surgery and transfusions. When straight lines were fitted to the data by the method of least squares, there was a significant difference in slope between the oozing and the nonoozing patients (t = 4.68; t for P 0.01 = 2.68). This indicated that increasing amounts of blood caused a greater depression of platelets in oozers than in nonoozers. Despite this tendency, severe thrombocytopenia was not present in the oozing patients; the lowest platelet count in an oozing patient during surgery was 112,000/c.mm., and the average was 196,000 (Table II).

Plasma Citrate Concentration.—The plasma citrate concentration was increased in proportion to the amount and rate of blood replacement.[21] Although

the plasma citrate level was elevated in all but two of the patients who oozed, it was not possible to establish a direct relationship between oozing and plasma citrate levels (Fig. 3). Oozing did not always coincide with peak plasma citrate levels. It did not always stop even when plasma citrate levels fell below 10 mg. per cent, and sometimes ceased without an appreciable drop in the plasma citrate. Plasma citrate levels of 30 or 40 mg. per cent were not uncommon, and levels as high as 79.5 mg. per cent were observed in the absence of oozing. One gram of calcium, either as the chloride or gluconate was administered for each two units of transfused blood.

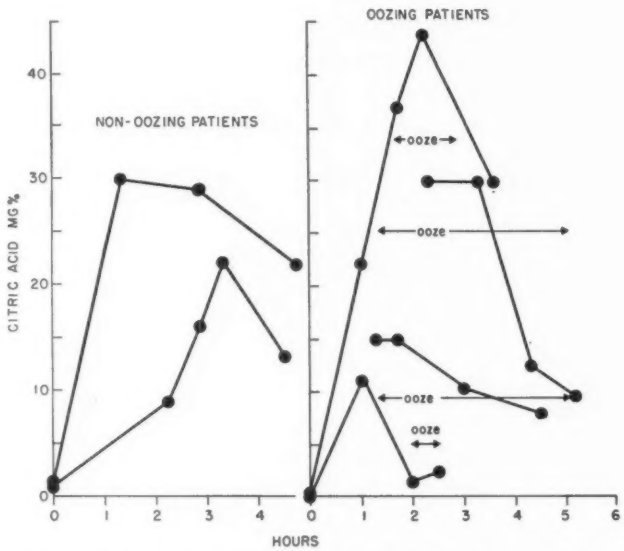

Fig. 3.—Plasma citrate concentration during surgery and multiple transfusions in selected nonoozing and oozing patients.

Fibrinolytic Activity.—Samples were considered to have activity only when complete lysis occurred in less than 24 hours for the whole blood samples, and in less than 10 hours for the euglobulin samples. Euglobulin activity was frequently observed when a simultaneous sample did not show whole clot lysis. When whole clot lysis was present, the euglobulin fraction always showed some fibrinolytic activity.

The relationship between the type of operation and the occurrence of fibrinolytic activity is shown in Table I. Patients undergoing hepatic lobectomy had the highest incidence of fibrinolytic activity.

The 40 patients previously reported[22] are referred to as Series I and were studied between September, 1955, and March, 1956. None of these patients had fibrinolytic activity prior to surgery. Seven of them oozed during surgery. Whole clot lysis occurred in 5 of these oozing patients, but was not determined in the other 2. Six of the oozing patients had fibrinolytic activity of the euglobulin fraction (Fig. 4, Table II). In contrast to the high incidence of fibrinolytic

activity observed in the oozing patients, only 4 of the 33 nonoozing patients showed whole clot lysis, with lysis times between 8 and 24 hours. These 4 patients also showed euglobulin activity (Fig. 4).

The 38 patients studied from March to July, 1956, Series II, did not show as definite a relationship between oozing and fibrinolysis. Of the 8 oozing patients only one had whole clot lysis, and 3 showed definite euglobulin activity (Fig. 4, Table II). Of the 30 nonoozing patients, one had 2-hour whole clot lysis during surgery, 3 showed euglobulin activity prior to surgery, and 10 had euglobulin activity during surgery (Fig. 4). The techniques employed were the same in both series with the following exceptions. In Series II, blood samples were obtained less frequently prior to the oozing episode and in most patients samples were not obtained during the actual oozing. In Series I, the mixture of patients' euglobulin fraction and fibrinogen was clotted with 50 units of thrombin, whereas in Series II, only one unit of thrombin was used. This change was made when it was found that the thrombin was contaminated with bovine plasminogen.[23]

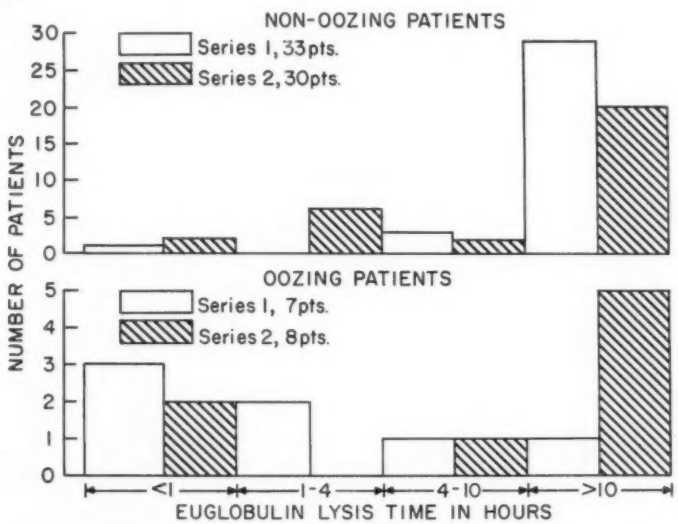

Fig. 4.—Euglobulin fibrinolytic activity in oozing and nonoozing patients.

When lytic activity was present, it was observed in one or more samples drawn close to the time of excessive bleeding. In 3 of the 9 oozing patients with significant fibrinolytic activity during surgery, no fibrinolytic activity was found in samples taken about one hour after closure. Two patients in Series II oozed just before or during closure, and no blood samples were taken until about an hour after closure. These postoperative samples showed fibrinolytic activity which was no longer present two hours later. Three patients who showed fibrinolytic activity while they were oozing during surgery, showed less activity in postoperative samples taken ½ to 3 hours after closure, and activity was no longer present in subsequent samples taken 3 to 6 hours later. In these patients, no apparent oozing occurred after closure. In one patient

(C. D.), oozing persisted for several hours after operation. Four hours postoperatively this patient showed fibrinolytic activity which was more marked than during surgery. Two hours later fibrinolytic activity had disappeared and bleeding was no longer observed.

Fibrinogen.—The fibrinogen concentration was below 170 mg. per cent in 5 and below 100 mg. per cent in 3 of the oozing patients. Two of the latter had had hepatic lobectomies (Table II). It is of interest that all of these were in Series I. All 5 had some fibrinolytic activity. It seems probable that a relationship exists between the two. Hypofibrinogenemia also developed in 4 nonoozing patients.* In one of them, a patient undergoing hepatic lobectomy, fibrinogen concentration fell below 100 mg. per cent, and in the other 3, who were undergoing other operations, it fell to slightly below 150 mg. per cent. Fibrinolytic activity was not detected in the latter 3 patients.

Several patients showed marked decreases in fibrinogen concentration during surgery but because the control concentration was high it did not fall to levels usually associated with bleeding. Fibrinogen fell more than 100 mg. per cent during surgery in patients who had no demonstrable fibrinolytic activity as well as in those with fibrinolytic activity. In some patients, the fall in fibrinogen could be attributed to transfusions, in which the patient's blood, with an abnormally high fibrinogen concentration, was replaced with bank blood containing a normal level of fibrinogen.

Prothrombin Time and Factors Influencing It.—The longest prothrombin time among the nonoozing patients was 18.8 seconds, compared to 19.0 seconds among the nonoozing patients. Reduction of prothrombin concentration was almost uniformly observed among the patients undergoing hepatic lobectomy, but values below 46 per cent were not reached until after surgery had been completed.[33] None of the other operative procedures resulted in less than 50 per cent prothrombin concentration. The proportion of patients with low prothrombin concentration was about equal in the oozing and nonoozing groups. Factor VII was not measured in all patients but, when determined, showed similar changes.

Factor V was low among the oozing patients more often than prothrombin. Furthermore, a decrease in factor V was found with significantly greater frequency among the oozing patients than among those who did not bleed excessively. (Chi square = 14.3; P 0.01 = 6.6). Fibrinolytic activity was present in all but one (T. A.) of the oozing patients who showed a drop in factor V and may have been responsible for destruction of this clotting factor.[31]

Prothrombin Consumption.—Three of the oozing patients (two of them women) showed less than 90 per cent consumption of prothrombin in one hour in samples obtained immediately prior to or early during surgery, although they had adequate platelet counts. It is possible that this abnormality accounts for the excessive surgical bleeding seen in these patients.[25]

*Fourteen patients are not included because determinations were made with the Parfentjev method.[24] When the fibrinogen concentration is low, this method gives results considerably below those obtained by the Cullen and Van Slyke method.

Shock, Transfusion Reactions, and Dextran Administration.—Shock and tissue hypoxia may cause derangements in the hemostatic mechanism either during operation or in the postoperative period. In this series there were 5 patients in frank shock. Only two of them oozed and had fibrinolytic activity. In the remaining 13 patients who exhibited oozing, significant hypotension during the operation or the postoperative period was not noted.

Although hemolytic transfusion reactions have been implicated as a cause of excessive surgical bleeding,[26] there is no evidence that any of our patients received incompatible blood. To eliminate subgroup incompatibility, direct cross-matching as well as indirect Coombs tests were done on all patients.

Two of the oozing patients received dextran during surgery. Although this plasma volume expander has been found to induce a hemorrhagic syndrome when given in large amounts to normal individuals,[27] dextran was not responsible for the initiation of excessive bleeding in our patients, since oozing was present before dextran was given.

TABLE III. CHANGES SHOWN BY NONOOZING AND OOZING PATIENTS

	NO CHANGE	INADEQUATE DATA	CHANGE IN SINGLE CATEGORY			
			LYSIS*	PROTHROM. FACTORS†	PLATS‡	TOTAL
Nonoozing	21	7	10	20	1	31
Oozing	1	1	0	3	0	3

Changes in Multiple Categories

	LYSIS FIBRIN.§	LYSIS PROTHROM. FACTORS	LYSIS FIBRIN. PROTHROM. FACTORS	LYSIS PROTHROM. CONS.‖ PROTHROM. FACTORS	PROTHROM. FACTORS PROTHROM. CONS.	PLATS. PROTHROM. FACTORS	TOTAL
Nonoozing	0	2	1	0	0	1	4
Oozing	1	4	2	1	2	0	10

*Euglobulin lysis time less than 10 hours.
†Prothrombin, factor V or VII below 70 per cent.
‡Platelet count under 100,000 c.mm.
§Less than 100 mg. per cent.
‖Less than 90 per cent in one hour.

Differences Between Oozing and Nonoozing Patients.—The results are summarized in Table III. The changes observed were classified in the following categories: (1) euglobulin fibrinolytic activity with lysis in less than 10 hours; (2) prothrombin, factor V, or factor VII below 70 per cent of normal; (3) fibrinogen concentration less than 100 mg. per cent; (4) platelet count less than 100,000/c.mm.; and (5) prothrombin consumption less than 90 per cent in one hour. The patients are divided into four groups: those showing no changes; those with incomplete data; those showing only one type of deficiency, that is, a change in only one category; and finally, patients showing multiple deficiencies or changes in more than one category. Among the nonoozing patients with adequate data, 21 showed no change in any of the factors studied, 31 showed changes in only a single category, and only 4 showed multiple changes. In contrast, only one of the oozing patients showed no change in any category, 4

showed a change in a single category only, and 9 showed changes in more than one category. If the 3 oozing patients with poor prothrombin consumption are omitted because this alone is presumably an adequate explanation of their bleeding, 3 oozing patients showed a change in a single category only and 7 showed multiple changes.

There proved to be significant differences between the distribution of oozers and nonoozers in the 3 categories (no change, single change, and multiple changes). (Chi square = 18.86, P 0.01 = 9.21.) The significance becomes even greater if the oozing patients with poor prothrombin consumption are included. Many more oozers are found in the multiple change category than one would expect if the distribution were the same for oozers and nonoozers. When a comparison is made between patients who had multiple changes and those who had changes in one category only, there is also a significant difference. (Chi square = 11.43, P 0.01 = 6.64.) Those patients who had a change in more than one category showed a significantly greater tendency to ooze.

DISCUSSION

Although the occurrence of fatal hemorrhage in obstetrics and surgery is rare, episodes of excessive bleeding during massive surgery are not uncommon. In this series, oozing occurred in 15 of 78 patients. Only 4 of the oozing patients received over 10 units of blood and in one of these the transfusions were the result rather than the cause of the excessive bleeding. Hence, massive transfusion was not a cause of oozing in most of the patients.

Thrombocytopenia following massive blood replacement has been reported by Stefanini and associates[1] and by Krevans and Jackson.[3] We have observed profound falls in the platelet count after less than 8 units of blood, and always after the administration of 10 or more units. The percentage reduction in count in our patients is approximately the same as in the patients studied by Krevans and Jackson.[3] However, only two of our patients had platelet counts below 100,000/c.mm. after transfusion. One explanation for the lack of thrombocytopenia in our cases is the high pretransfusion platelet count in many of our patients. Another is that Krevans and Jackson reported the lowest platelet count within 48 hours after transfusion, whereas we report the count within a few hours. The platelet count generally continues to fall for one or two days after blood replacement. Thrombocytopenia did not appear to be a significant factor in the etiology of oozing in our series, although there was a tendency for a greater depression of the platelet count among the oozers.

Although "citrate intoxication" has been discussed repeatedly since the advent of the modern blood bank, there has been little or no clinical study of the correlation between blood citrate levels and oozing. In this series, patients showed an oozing tendency with and without elevated blood citrate levels. Some patients with markedly elevated citrate levels did not ooze. There was no evidence from our studies that elevated citrate concentration affected blood clotting. Presumably, the administration of calcium salts and the mobilization of body calcium stores adequately maintained ionized calcium.

Our results suggest that two criteria should be observed in an attempt to assess the relationship between fibrinolytic activity and excessive surgical bleeding. First, samples should be drawn during the period of active bleeding, since fibrinolytic activity often disappears when oozing ceases. Second, frequent samples should be obtained. Because of the rapid fluctuations in fibrinolytic activity, a single sample may give misleading results.

The mechanisms which activate the fibrinolytic enzyme are not clearly understood. Stress,[28] shock,[29] and hypoxia[22] can activate fibrinolysin and may play a role in surgical cases.[28] Shock was not severe in most of our oozing patients. Hypoxia was considered to be the cause of fibrinolytic activity in two of the nonoozing patients in the first series.[22] Tissues, particularly the lung and uterus, are said to contain activators[30] which may be released into the circulation during surgical trauma. Fibrinolysin may interfere with hemostasis by lysis of clots or it may destroy some of the circulating blood clotting factors.[31]

Previous studies of surgical bleeding have consisted, for the most part, of case reports of patients with very severe hemorrhagic syndromes. A striking feature of some of these reports has been the failure of the patient's blood to clot even when thrombin was added.[5, 6, 10] This has been attributed to the absence of circulating fibrinogen. It should be remembered that incoagulable blood may result from rapid lysis of newly formed fibrin strands. In vitro lysis of fibrin occurs more rapidly than does destruction of the fibrinogen in blood prevented from clotting by citrate or oxalate, so that rapid whole clot lysis may occur when the fibrinogen concentration is only moderately low. For example, in one sample in a severely oozing patient not included in this series, the clot had lysed completely in less than 20 minutes. If inadequately observed, this might have been mistaken for complete absence of clot formation and interpreted as afibrinogenemia; yet, a simultaneous oxalated sample had a fibrinogen concentration of at least 175 mg. per cent.

Fibrinogen concentrations below 100 mg. per cent were observed in only 3 of the oozing patients, all of whom had fibrinolytic activity. In cases reported by others, hypofibrinogenemia in surgery or obstetrics was often associated with demonstrable fibrinolytic enzyme, but, in some, fibrinolytic activity was not detected.[12, 13] In the latter cases, intravascular coagulation has been implicated as a cause of hypofibrinogenemia.[12, 32] The failure to detect activity could be ascribed to lack of sensitivity of the method employed,[12] or to disappearance of activity at the time of sampling.

Low fibrinogen concentration, rather than complete afibrinogenemia has been observed in most of the bleeding patients described by Coon and Hodgson,[9] Hodgkinson and associates,[10] and Phillips, Rowley, and Habif.[11] Although patients with congenital afibrinogenemia have fairly adequate hemostasis in the face of day-to-day trauma,[25] Phillips and associates[11] observed operative bleeding in patients with concentrations of fibrinogen as high as 125 to 175 mg. per cent and suggest that a higher level of fibrinogen is needed for hemostasis during surgery.

Decreased concentrations of prothrombin, factor V, and factor VII were found during surgery in patients who did not ooze, as well as in some who had excessive bleeding. These factors may have been destroyed by the fibrinolytic enzyme in some cases, but similar reductions were observed in patients in whom fibrinolytic activity was not detected. Since these clotting factors are short-lived,[25] this fall in concentration may represent utilization without adequate replacement. The liver is necessary for normal production of prothrombin and factors V and VII. With partial hepatectomy, factor V reaches its lowest level within 12 hours after surgery; prothrombin and factor VII fall more slowly, but are generally already somewhat reduced by the end of surgery.[33] It is possible that the minor decreases in these factors observed in some patients undergoing operations other than hepatic lobectomy may be the result of a temporary decrease in hepatic production.

We conclude that no single factor is responsible for excessive bleeding during surgery. In fact, there was a statistically significant relationship between the occurrence of excessive bleeding and the presence of multiple changes in the factors studied. Fibrinolytic activity was the change most frequently associated with oozing, but fibrinolysis was sometimes present without obvious oozing and was occasionally absent in oozers. In most of the patients who oozed excessively, some change in addition to fibrinolysis was noted, usually a decrease in fibrinogen, prothrombin, factor V, or factor VII. Although these clotting factors may have been destroyed by the fibrinolytic enzyme, similar decreases were observed in patients without detectable fibrinolytic activity.

Multiple deficiencies were also found in two patients with severe oozing, not included in the series because of lack of control data. Both whole blood and euglobulin fibrinolytic activity were very pronounced in these patients. The fibrinogen level was low normal, but prothrombin, factor V, and factor VII, and platelet levels were below normal.

It is difficult to assess therapeutic measures because both lysis and oozing may cease without treatment. We have used lyophilized fresh (antihemophilic) plasma largely because many of the samples tested had very high antifibrinolytic activity. It also contains prothrombin, factor VII, and fibrinogen in approximately normal concentration, but there is a subnormal concentration of factor V. Fresh whole blood collected in plastic bags is recommended when thrombocytopenia is present, and fibrinogen (fraction I) should be given when severe hypofibrinogenemia occurs.

SUMMARY

Blood clotting factors and fibrinolysis were studied in 78 patients before, during, and after major surgery.

Fifteen patients oozed excessively during surgery. Oozing could not be ascribed to massive blood replacement, and in none of the oozing patients was the platelet count below 100,000/c.mm. Plasma citrate concentration bore no relationship to the occurrence of oozing.

Fibrinolytic activity of the euglobulin fraction was found during or shortly after surgery in nine of the 15 oozing patients. Active whole clot lysis was

observed in 6 cases. Fourteen of the 63 nonoozing patients had euglobulin fibrinolytic activity and 5 had whole clot lysis. Fibrinogen concentration fell below 100 mg. per cent in only 3 of the oozing patients, 2 of whom were undergoing hepatic lobectomy. Decreases in prothrombin and factor VII concentrations during surgery occurred with equal frequency among oozers and nonoozers, whereas decreases in factor V occurred more frequently in oozing patients. Values below 50 per cent of normal were only observed in patients undergoing hepatic lobectomy.

All but one of 14 oozing patients showed a change in at least one of the factors measured. Three had poor prothrombin consumption before surgery, 3 had prothrombin or factor V concentrations below 70 per cent, and the remaining 7 had fibrinolytic activity plus some other change. The correlation between oozing and the occurrence of multiple changes was statistically significant.

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STUDIES IN IRON TRANSPORTATION AND METABOLISM

X. Long-Term Iron Overload in Dogs

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INTRODUCTION

HEMOCHROMATOSIS, characterized by pigmentary cirrhosis, pancreatic fibrosis with diabetes, and extensive parenchymal organ infiltration with iron, has not been produced in experimental animals despite attempts by many investigators. In these unsuccessful efforts a wide variety of animal species, many forms of iron compounds, and multiple routes of administration have been used. Many attempts to produce hemochromatosis experimentally may have failed because the amounts of iron given were too small or the subsequent period of observation too brief to allow the changes of this disease to occur. However, patients given multiple transfusions[1, 2] or prolonged excessive oral doses of medicinal iron,[3-6] and Bantu natives[7-10] ingesting large amounts of dietary iron do develop hemosiderosis and may show cirrhosis of the liver and rarely diabetes. That conditions other than idiopathic hemochromatosis are associated with increased tissue iron stores and organ damage in human beings, makes more remarkable the failure to produce similar changes in animals. An examination of previous experimental attempts to produce hemochromatosis seems indicated in order to view the problem more clearly and to evaluate the results we shall present in this study.

REVIEW OF LITERATURE

Among the most complete studies to delineate the pathogenesis of hemochromatosis were those of Polson,[11-13] who conducted both acute and long-term experiments on the fate of colloidal iron administered intravenously to rabbits. Using relatively small doses of iron (0.1 to 0.3 Gm./Kg.) and observing the animals for only a year, he found the injected iron was deposited initially in largest amounts in the reticuloendothelial elements of liver, spleen, lymph nodes, and bone marrow. Small amounts of hemosiderin were present as emboli in pulmonary and glomerular capillaries and in scattered macrophages elsewhere in the body. Little iron was found in hepatic parenchymal cells until redistribution from Kupffer cells occurred 6 to 8 months after the beginning of injections. Though considerable aggregation of iron granules occurred, there was little cellular reaction to its presence, and nothing

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similar to the fibrosis and cirrhosis of hemochromatosis was produced. This same investigator injected colloidal iron in doses of 0.13 to 0.33 Gm./Kg. subcutaneously and intraperitoneally into rabbits for periods as long as 4 years with essentially the same histologic results.

Careful experiments similar to those of Polson were reported in 1930 by Cappell[14] who injected saccharated iron oxide in 550 mg./Kg. doses into mice and rats which were thereafter observed for periods as long as 14 months. With minor exceptions, his results confirmed Polson's work and led him to conclude that "nothing analogous to the lesions of hemochromatosis in the human subject has been observed in the organs of animals subjected to excess iron over prolonged periods."

Andersson[15] injected a preparation similar to saccharated iron oxide intravenously into rabbits and followed 4 animals for as long as one year. Doses of iron in the range of 0.3 to 0.35 Gm./Kg. produced no cirrhosis or other organ damage.

Extensive studies using intravenously administered saccharated iron oxide or subcutaneous and intraperitoneal injections of "ferric hydroxide ferrous ascorbate" in mice, rats, rabbits, and guinea pigs were performed by Nissim.[16] He achieved doses of iron as high as 2.16 Gm./Kg. in mice and observed them until death occurred "months" after the last injection. Guinea pigs were followed for approximately 3 months from the beginning of subcutaneous injections of "ferric hydroxide ferrous ascorbate" in doses as high as 1 Gm. of iron per kilogram of body weight. While tissue damage was produced as evidenced by hemorrhagic embolic phenomena in the lungs, patchy parenchymal damage in the liver, and adrenal hemorrhages, no cirrhosis of the liver or pancreatic fibrosis was observed. This author emphasized that the distribution of iron deposits achieved and the organ damage produced depended upon the type of preparation used.

In as yet unpublished observations on the long-term toxicity of an iron-dextran complex, Golberg[17] reports no evidence of cirrhosis or organ damage from iron deposits resulting from doses as high as 1.65 Gm./Kg. in rats or rabbits observed more than 40 weeks.

Hemoglobin iron liberated from the animal's own red cells by hemolytic agents[18-24] or from nonviable transfused cells has been used by several investigators in attempts to produce hemochromatosis in experimental animals. Among the earliest of these studies were those of Rous and Oliver[25] who transfused rabbits with citrated whole blood daily for periods up to 6½ months. They produced abundant hemosiderin pigmentation of reticuloendothelial cells but no cirrhosis; their conclusion was "that hemosiderin, when enclosed in living cells, has no noteworthy stimulative effect on connective tissue."

Since the excess iron in idiopathic hemochromatosis and in cytosiderosis of Bantu natives must ultimately be derived from increased absorption from the gastrointestinal tract,[9, 10, 26, 27] many investigators have attempted to increase iron absorption in animals. While it has been repeatedly shown that some form of block to uncontrolled iron absorption exists in several animal species as well as in man, Hegsted and co-workers[28] demonstrated in rats, mice, guinea pigs, and chickens that if sufficient iron is added to a normal diet all species will absorb large amounts of iron. They also showed[29, 30] that even greater amounts of iron will be absorbed if a corn-grit diet low in phosphorus or high in certain amino acids is fed. When one increases the absorption of iron by these means, iron is deposited primarily in the liver and spleen. Its distribution in parenchymal as well as in reticuloendothelial cells is somewhat suggestive of that in hemochromatosis, but there is no associated fibrosis or cirrhosis. Wyatt and Howell[31] extended the corn-grit dietary experiment of Hegsted and co-workers and added other forms of tissue injury produced by castration or feeding of copper salts. Excessive iron storage in the liver was again produced, but no evidence of progressive parenchymal fibrosis was observed. Likewise, Rather[79] fed ferric citrate alone or combined with a low-protein diet to rats observed as long as 20 months. He found no fibrosis of the liver or pancreas despite large hepatic hemosiderin deposits.

Increased absorption of iron added to the diets of golden hamsters has been described[32] when emulsifying agents are also added. Siderosis of the liver is thereby produced, but no convincing evidence of cirrhosis or other changes of hemochromatosis have been described.

Following ligation of the pancreatic ducts in cats, fibrosis of the pancreas and siderosis of the liver, spleen, and other organs occurs as first described by Taylor, Stiven, and Reid[33]

TABLE IA. ATTEMPTS TO PRODUCE IRON OVERLOAD IN EXPERIMENTAL ANIMALS. IRON INTRODUCED PARENTERALLY

AUTHOR	EXPERIMENTAL ANIMAL	ROUTE OF ADMINISTRATION	Fe COMPOUND AND DOSE	DURATION OF OBSERVATIONS	COMMENT
Polson[11-13]	Rabbit	Intravenous	Colloidal Iron (0.1-0.3 Gm./Kg.)	14 mo.	Iron was confined initially to reticuloendothelial cells with eventual redistribution to hepatic parenchymal cells (after 6-8 mo.) without fibrosis or cirrhosis
	Rabbit	Intramuscular Intraperitoneal	Colloidal Iron (0.13-0.33 Gm./Kg.)	4 yr.	Essentially the same results as with intravenous administration
Cappell[14]	Mouse Rat	Intravenous	Saccharated Iron Oxide (0.55 Gm./Kg.)	14 mo.	Confirmed Polson's observations
Andersson[15]	Rabbit	Intravenous	Saccharated Iron Oxide (0.3-0.35 Gm./Kg.)	1 yr.	Confirmed Polson's observations
Nissim[16]	Mouse	Intravenous	Saccharated Iron Oxide (1.8-2.16 Gm./Kg.)	5 mo.	
	Rat	Intravenous	Saccharated Iron Oxide (0.6-1.0 Gm./Kg.)	6-12 wk.	Hemorrhagic embolic phenomena in the lungs, patchy parenchymal damage in the liver, and adrenal hemorrhages were produced. However, no cirrhosis of the liver or pancreatic fibrosis was seen
	Rabbit	Intravenous	Saccharated Iron Oxide (0.09-0.54 Gm./Kg.)	4-6 wk.	
	Guinea pig	Intraperitoneal and subcutaneous	Ferric hydroxide Ferrous Ascorbate (0.17-1.0 Gm./Kg.)	3 mo.	
Golberg[17]	Rat Rabbit	Intramuscular	Iron Dextran (1.65 Gm./Kg.)	10 mo.	No evidence of cirrhosis or organ damage despite massive hemosiderosis
Finch[42, 43]	Dog	Intravenous	Iron Ascorbate Gelatin (1.44 to 4.20 Gm. Fe)	2 yr.	No cirrhosis or organ damage
	Dog	Intravenous	Red Blood Cells (1.76 Gm. Fe)	15 mo.	Iron was deposited mainly in the reticuloendothelial cells; the distribution was comparable to that in dogs given iron ascorbate gelatin. No tissue damage
	Dog	Intravenous	Saccharated Iron Oxide (2 Gm./Kg.)	2 yr.	Dogs developed hepatomegaly, persistent ascites, and "other evidence of liver failure" but minimal fibrosis
Rous and Oliver[25]	Rabbit	Intravenous	Red Blood Cells (Approx. 1 Gm. Fe)	3-6½ mo.	Transfused citrated whole blood and produced abundant hemosiderin pigmentation of reticuloendothelial cells but no cirrhosis
Krumbhaar and Chanutin[44]	Dog	Intravenous	Red Blood Cells (2.5-5.0 Gm. Fe)	6 mo.	Transfused whole citrated blood (5-10 L.) and produced hemosiderosis confined mainly to the reticuloendothelial cells without fibrosis
Platzer and Young[2]	Dog	Intravenous	Red Blood Cells (0.14-0.22 Gm./Kg.)	3-11 mo.	Produced hemosiderosis without fibrosis or other abnormalities

TABLE IB. ATTEMPTS TO PRODUCE IRON OVERLOAD IN EXPERIMENTAL ANIMALS. IRON INTRODUCED ORALLY

AUTHOR	EXPERIMENTAL ANIMAL	ROUTE OF ADMINISTRATION	Fe COMPOUND AND DOSE	DURATION OF OBSERVATIONS	COMMENT
Hegsted et al.[28]	Rat Mouse Chicken Guinea pig	Oral	2-12% Fe-citrate alone or with corn grits	27-81 days	When sufficient iron was added to a normal diet increased absorption occurred. Even greater absorption occurred if corn grit diet low in phosphorus was fed
Wyatt and Howell[31]	Rat	Oral	2% Fe-citrate plus corn grits	50-150 days	Extended corn grit diet experiments of Hegsted and added tissue injury in the form of castration and feeding copper salts. Produced increased hepatic iron storage without fibrosis
Rather[79]	Rat	Oral	6% Fe-citrate alone or with a low-protein diet	20 mo.	Produced large amounts of hemosiderin in the liver without fibrosis of liver or pancreas
Finch[42]	Dog	Oral	2% Fe-citrate plus corn grits	2-6 mo.	Produced marked hepatic parenchymal hemosiderosis. No cirrhosis of the liver; no pancreatic or splenic fibrosis
Wissler et al.[32]	Hamster	Oral	--	2-5 mo.	Polyoxyethylene sorbitan monolaurate (Tween 20) given orally produced increased iron absorption but no changes of hemochromatosis
Taylor et al.[33]	Cat	Oral	--	55-199 days	Ligation of pancreatic ducts led to pancreatic fibrosis and increased iron absorption. Hemosiderosis was accentuated by dietary iron supplements. No hemochromatosis seen
Gilman et al.[34]	Cat	Oral	--	--	Repeated and confirmed the observations of Taylor et al.
Kinney et al.[45]	Dog	Oral	--	2-5 mo.	Confirmed observations of Taylor et al. in dogs. No evidence of cirrhosis of the liver or hemochromatosis
Kinney et al.[35]	Rat Mouse	Oral	2% Fe-citrate plus DL-ethionine	28 days	Ethionine produced damage to the pancreas, liver, and testes plus increased iron absorption. When iron was added to the diet, lesions superficially like those of hemochromatosis appeared
Hall and MacKay[36]	Rabbit	--	--	21-105 days	Copper acetate given by mouth produced hepatic necrosis and eventual cirrhosis plus hemofuscin deposition with slight degrees of hemosiderosis
Mallory and Parker[37]	Rabbit	--	--	5 mo.	Similar experiments to Hall and MacKay's using subcutaneous injections of copper
Herbut et al.[41]	Rabbit	--	--	14-102 days	Alloxan produced pancreatic and hepatic necrosis with eventual fibrosis plus slight hemosiderosis of liver and spleen

and confirmed by the Gillmans.[34] The siderosis was further accentuated by feeding a diet high in iron. Except for the duct-ligated pancreas, no fibrosis accompanied the iron so deposited in the tissues.

Ethionine administration produces damage to the pancreas, liver, and testes plus increased iron absorption. When iron is added to the diets of rats or mice so treated, siderosis of the liver and other organs plus the ethionine-induced damage produces lesions superficially like those of hemochromatosis.[35] Likewise, copper given by mouth[36] or subcutaneously[37] to rabbits results in necrosis and eventual cirrhosis of the liver with slight degrees of siderosis. While disturbances in copper metabolism have been described in idiopathic hemochromatosis[38-40] there is little evidence to suggest that copper plays an etiologic role in its production. Similarly, the toxic effects of alloxan in producing pancreatic and hepatic necrosis with eventual fibrosis and slight hemosiderin deposition have occasioned the use of this drug in unsuccessful attempts to produce hemochromatosis in rabbits.[41]

Most of the above-mentioned techniques have also been applied in attempts to produce hemochromatosis in the dog. In a comprehensive study of iron storage, Finch[42] injected 1.44 to 4.2 Gm. of iron as iron-ascorbate gelatin intravenously into 4 dogs which were then observed for periods as long as 2 years. Despite increases of iron concentration in the liver to approximately 25 times normal, no cirrhosis or other organ damage was produced. Transfusions of nonviable red cells to a total of 1.76 Gm. of iron were given to another dog sacrificed after 15 months. The distribution of iron from hemoglobin breakdown was entirely comparable to that produced by the iron-ascorbate gelatin and no tissue damage was mentioned other than scars involving wedges of renal tubular cells. Five dogs were fed a corn-grit diet supplemented by 2 per cent ferric citrate for periods of from 60 to 175 days. As had been demonstrated in rats and mice on a similar diet, iron concentrations in the livers of these dogs increased on the average to approximately 25 times normal while in the spleens there was a tenfold increase. Distribution of iron tended to be greater in parenchymal cells here than in dogs given iron parenterally, though reticuloendothelial deposits were also great. Again, no evidence of cirrhosis of the liver or fibrosis of pancreas or spleen was found. In a subsequent paper Finch[43] cites his experience with the intravenous injection of 2 Gm. of iron per kilogram of weight as saccharated iron oxide into normal dogs which were observed for two years. These animals developed hepatomegaly, persistent ascites, and "other evidence of liver failure," but minimal fibrosis in proximity to siderotic nodules in the liver was the only change resembling the cirrhosis of idiopathic hemochromatosis.

Transfusions of citrated whole blood totaling 5 to 10 L. were given to dogs over a 6-month period by Krumbhaar and Chanutin.[44] When sacrificed, the animals showed marked congestion of all organs with hemosiderin pigmentation confined to macrophages in liver, spleen, lymph nodes, and other organs; there was no fibrosis. Likewise, Platzer and associates[2] transfused two dogs to iron levels of 0.14 and 0.22 Gm./Kg. before sacrifice at 3 and 11 months. They reported that with the exception of the polygonal cells of the liver, all visible iron was stored in reticuloendothelial cells, and neither fibrosis nor other abnormalities were noted in any organ. Pancreatic duct ligation in dogs[45] resulted in moderate siderosis of liver and spleen after 5 months but did not produce cirrhosis or other changes of hemochromatosis. These observations are summarized in Tables Ia and Ib.

The experimental attempts to produce hemochromatosis in animals summarized above clearly point up the relative ease with which iron can be introduced into the organism as well as the great difficulty of achieving parenchymal localization of the iron and of producing the fibrosis of liver, spleen, and pancreas which are hallmarks of idiopathic hemochromatosis in human beings. In many instances the concentration of iron achieved seemed too small or the period of observation too brief to be sure that the lesions of this disease had time to appear. With these objections in mind the following studies were undertaken.

Normal dogs were given iron as saccharated iron oxide or as hemoglobin from transfused red blood cells in doses of 0.5 to 1.0 Gm./Kg. body weight. These

dogs were then observed for periods as long as 7 years. During the period of observation, measurements of hepatic function were performed together with evaluation of hematologic status and changes in iron metabolism. Repeated biopsies of liver, spleen, pancreas, and other organs were made for comparison with the histologic findings at autopsy; by these means the initial deposition, redistribution, and cellular reaction to iron deposits were studied. Chemical estimates of the iron content of various tissues were performed to quantitate iron deposition. Despite concentrations of iron chosen to correspond to those found in human hemochromatosis, no findings suggestive of this disease were detectable even after 7 years. Our failure to produce hemochromatosis in dogs, plus the similar experience of many investigators using other animals, raises the question whether increased iron deposits by themselves eventually produce the changes of hemochromatosis or whether additional factors must be present. This question is considered in the following discussion, though no definite conclusions can be achieved with the information now available.

MATERIALS AND METHODS

Healthy, full-grown, mongrel dogs weighing 5.9 to 17.5 kilograms were used. The animals were housed in individual cages, and were fed commercial dog chow. During an initial period of observation the animals were given hexylresorcinol as a vermifuge. At intervals throughout the study the feces were tested for occult blood by Gregerson's benzidine method[46] and whenever blood was found, the vermifuge treatment was repeated. Weights were obtained at frequent intervals.

Red cell counts were made at weekly intervals at first, and less frequently during the later phases of the study. Trenner red cell pipettes and counting chambers certified by the United States Bureau of Standards were used. Initially, hemoglobin was determined by the oxyhemoglobin method described by Evelyn[47] and in the last year of studies by the cyanmethemoglobin technique.[48] Packed red cell volume was determined from arterial or venous blood. Serum iron was measured by a method previously described.[49] The iron binding capacity of serum was measured by the method of Rath and Finch[50] or by that of Ventura.[51] Bromsulphalein retention as described by Gaebler[52] and serum bilirubin by Watson's modification of Evelyn and Malloy's method[53] were determined at 2-week intervals for 3 months and less frequently thereafter. Fractional serum protein determinations were performed by Weichselbaum's method.[54] Several specimens of serum were analyzed by paper electrophoretic methods[55] and scanned with a Spinco Analytrol. Fasting blood sugar determinations were done by a modified Nelson technique[56] at approximately monthly intervals for 6 months, then less frequently. Oral glucose tolerance tests as done by Fajans and associates[57] were performed on 3 animals shortly before sacrifice. Absorption of radioactive iron was measured in 3 dogs.[58] The utilization of intravenous tracer doses of radioactive iron was also studied.[59]

A single lot of saccharated iron oxide* was injected intravenously into 4 dogs over a 6- to 10-week period. In order to avoid perivenous infiltration of the iron solution, the injection was begun with isotonic saline, which was allowed to flow into the saphenous vein from a small elevated reservoir. As soon as the saline was flowing freely, the desired amount of Ferrivenin (5 to 15 ml. containing 100 to 300 mg. of iron) was added. The last traces were washed into the vein by addition of saline to the reservoir. Injections were given 5 times a week until a level of 0.5 Gm. or 1.0 Gm./Kg. was reached.

Five dogs received transfusions of blood from compatible donors (determined by cross matching initially and after the third transfusion). At first the dogs were given washed red cells suspended in saline to about half the original whole blood volume. After the sudden death of 3 of the dogs, whole blood was substituted for washed red cells because death

*Ferrivenin, Lot No. 10369, Benger Laboratories, Limited, Holmes Chapel, Cheshire, England.

was believed to have been due to a rapid increase in concentration of erythrocytes in the circulation as the saline diffused out. When whole blood transfusions were substituted, sodium citrate rather than A.C.D. solution was used as a preservative and the blood was stored for 7 days before transfusion. These measures allowed more rapid red cell breakdown and thereby relieved the load on the recipient's circulation. As a further precaution, transfusions of 200 ml. of whole blood were given 3 times a week rather than a transfusion of the washed cells from 800 ml. of blood once a week.

Transfusions to the two surviving dogs were continued for 6 to 10 months until an iron concentration of 0.5 Gm. per kilogram of body weight was attained. This iron level and those achieved with saccharated iron oxide were chosen to correspond to the concentrations of iron in patients with idiopathic hemochromatosis.

Laparotomies were performed under Nembutal anesthesia on 6 dogs before beginning iron injections. Wedge biopsies were obtained of liver, spleen, pancreas, kidney, a mesenteric lymph node, muscle, and skin. Measurement of the iron concentration of the liver section and microscopic examination of all these tissues were made. Within a month after completion of Ferrivenin injections a skin biopsy for iron analysis and histologic study was taken from the inner aspect of the thigh. Bone marrow samples were obtained by aspiration from the iliac crest or proximal femoral epiphysis. At approximately 6 months and again 3 years after the start of the experiment, laparotomies were performed and biopsies were taken of the organs previously mentioned. At death or sacrifice autopsies were performed as soon as feasible, usually within 3 hours of death.* Samples of tissue were obtained for histologic study and iron analysis from all the major organs at autopsy. All tissue samples were weighed within a short time of removal, the excess blood blotted off, and they were then wet-ashed with concentrated sulfuric acid. After digestion and dilution, the iron content was determined colorimetrically using the potassium thiocyanate (KSCN) method as described for serum iron determinations. Sections for histologic study were fixed in 10 per cent neutral formalin and Zenker's acetic solutions and stained with hematoxylin and eosin, and for iron with a modified Gomori stain,[60] counterstained with Mallory's basic fuschin. Selected sections were stained with Masson's connective tissue stain, methylene blue, periodic acid-Schiff, acid-fast, and Sudan IV stains.

RESULTS

Survival.—Of the 4 dogs which received saccharated iron oxide, two dogs (6 and 18) which had received 1.0 Gm. of iron per kilogram of weight were living and in good health at the time of sacrifice 81 and 84 months after the start of the experiment. One of the remaining animals that received Ferrivenin (17) died of intestinal obstruction due to biopsy-induced adhesions after 52 months, and the other dog (15) died in a laboratory accident 6 years after the onset of studies.

Three of the dogs receiving red cell transfusions died within a month of starting transfusions. The two animals that completed their course of transfusions were followed for 6 and 7 years. Dog 10 died in the same laboratory accident as Dog 15. Dog 20 was sacrificed after 84 months of the experiment. The ages of the dogs at death are unknown.

Reactions.—There was remarkably little reaction to single injections of large doses of saccharated iron oxide or to prolonged tissue iron accumulation. Weight was maintained or increased in all animals; appearance, vigor, and appetite remained good throughout the experiment. Only 3 immediate reactions of vomiting or diarrhea were seen in more than 150 injections of Ferrivenin. In

*Two dogs (10 and 15) died at night when a steam pipe burst in their quarters; they were not autopsied until the next day when the accident was discovered. Considerable postmortem autolysis occurred in the tissues of these animals, but the distribution of iron and lack of fibrous reaction in the various tissues seemed in every way comparable to the findings in the other dogs.

one dog, accidental perivenous infiltration of the iron solution resulted in a 2 cm. clean, flat-based ulcer on the leg which healed completely within 10 days. Fibrosis of the veins that occurred in most dogs toward the end of the course of injections was believed to be due more to the trauma of multiple venepunctures than to any sclerosing action of the iron solution.

Transfusions of washed red cells suspended in saline were poorly tolerated and led to the death of 3 dogs. The exact cause of death is not known but premonitory signs of vomiting and diarrhea occurred shortly after completion of transfusions. Two of the dogs (13 and 19) were later found dead in their cages, but in the third animal (Dog 12) the events leading to death were observed. Despite normal temperature, pulse, and breath sounds, there was progression to an involuntary running reflex, paralysis of the forelegs, and finally rhythmic convulsions. Adrenalin, oxygen administration, and phlebotomy were ineffective in reversing the reaction. Neither hemoglobinuria nor anuria was found; incoagulability of the blood was not present; and blood samples crossmatched with those of the donor dogs revealed no agglutination or hemolysis. Furthermore, autopsy examination revealed only marked congestion of all organs with no cause of death determined.

When whole blood preserved with 4 per cent sodium citrate was substituted for the A.C.D.-preserved, saline-suspended, washed red cells no further untoward reactions were noted in 120 transfusions. No evidence of gross hemolytic reactions was noted following any transfusion.

Unrelated to the iron administration was the occurrence in Dog 17 of a severe upper respiratory infection thought to be distemper. The infection was treated with penicillin and Aureomycin with complete recovery within 5 days. This animal, following its second laparotomy, had a complete wound dehiscence with herniation of the bowel and spleen. Early repair and vigorous antibiotic therapy resulted in prompt closure of the wound and complete healing, though the dog died several years later of intestinal obstruction due to peritoneal adhesions.

Hematologic Response.—Analysis of the red cell count, hemoglobin, and packed cell volume determinations reveals no marked variations from normal in the dogs given saccharated iron oxide. Dog 6 is representative of this group (Fig. 1). In all cases there was a moderate rise in the red count accompanied by a less distinct rise in hemoglobin and hematocrit 2 to 3 weeks after the initial administration of Ferrivenin. A marked fall of all blood values was noted in Dog 18 toward the end of the iron injections and similar but less marked patterns were seen for Dogs 6 and 15. In all of these dogs complete restoration to normal was noted within 1 to 3 months after injections were completed. Dog 17 showed little fall in any of the blood values during the injection period, but two months later had a definite decrease in all parameters with further accentuation at the time of an intercurrent bout of distemper. Within a month, recovery was complete. Subsequent determinations revealed only moderate fluctuations of blood counts. Since these dogs had normal blood values initially, there was no reason to expect any marked rise in red cell elements from iron administration. On the other hand, the fall noted in some instances shows a variable

temporal relationship with the iron injections and it is impossible to determine with the data available the mechanism or causal relationship to iron administration.

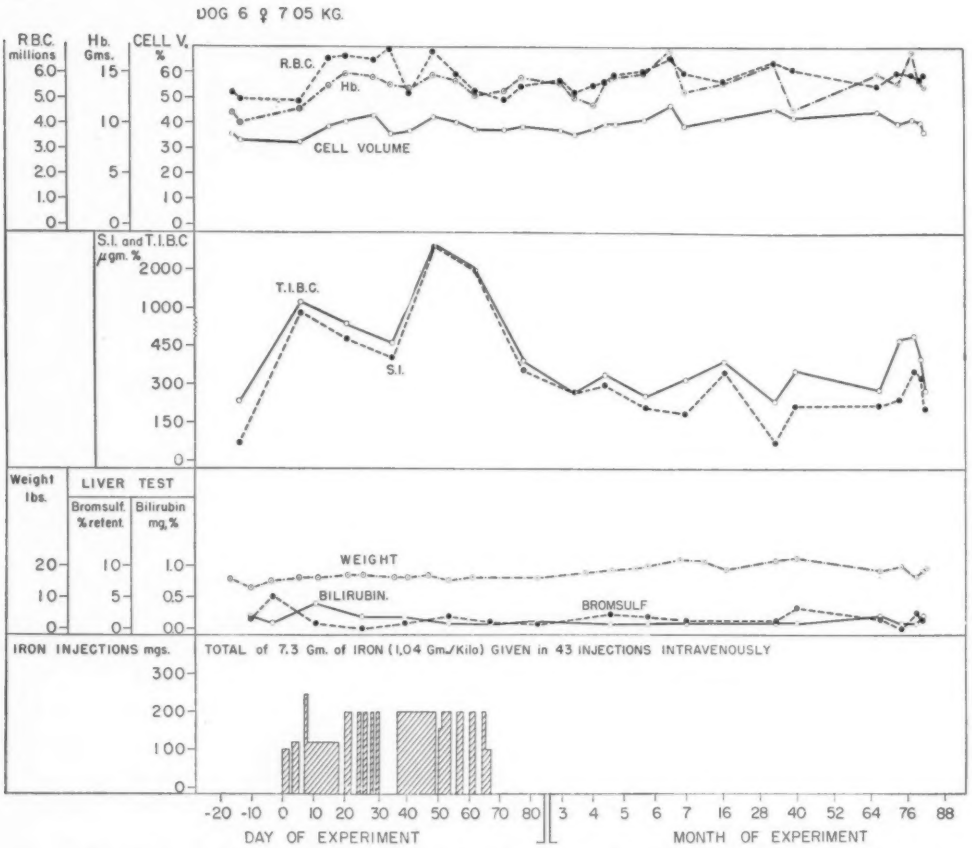


Fig. 1.—Effect of iron overload from injections of Ferrivenin on the blood counts, serum iron, body weight, and liver function of a dog observed 7 years. The amounts of iron injected intravenously are shown at the bottom of the chart.

A severe plethora was produced in all the transfused dogs. In the two dogs which received the greatest number of transfusions red cell counts reached levels greater than 15 million. Hemoglobin values were correspondingly elevated to peak values exceeding 26 Gm. per cent. Packed cell volumes were also elevated. These findings are shown graphically for Dog 10 in Fig. 2. Within a month following the last transfusion into Dog 10, the red cell count fell from 13 million to 6.5 million red cells per cubic millimeter and there were corresponding decreases in cell volume and hemoglobin values. A similar, but less well documented, fall to subnormal values occurred in Dog 20. There was no evidence of external blood loss; the anemia seemed most likely to be due to either a profound depression of the bone marrow secondary to prolonged transfusions, or an increased capacity for red cell breakdown by the reticuloendothelial system, or to a combination of both factors. A similar phenomenon was reported

by Krumbhaar and Chanutin[44] though they noted its occurrence during the course of transfusions and found it to be unaffected by splenectomy.

Bone marrow aspirations were done on the dogs given saccharated iron oxide approximately one month after the course of injections. Except for slight hypocellularity of both myeloid and erythroid elements in Dogs 15 and 18 and large granular aggregates of hemosiderin in all animals, no abnormalities were

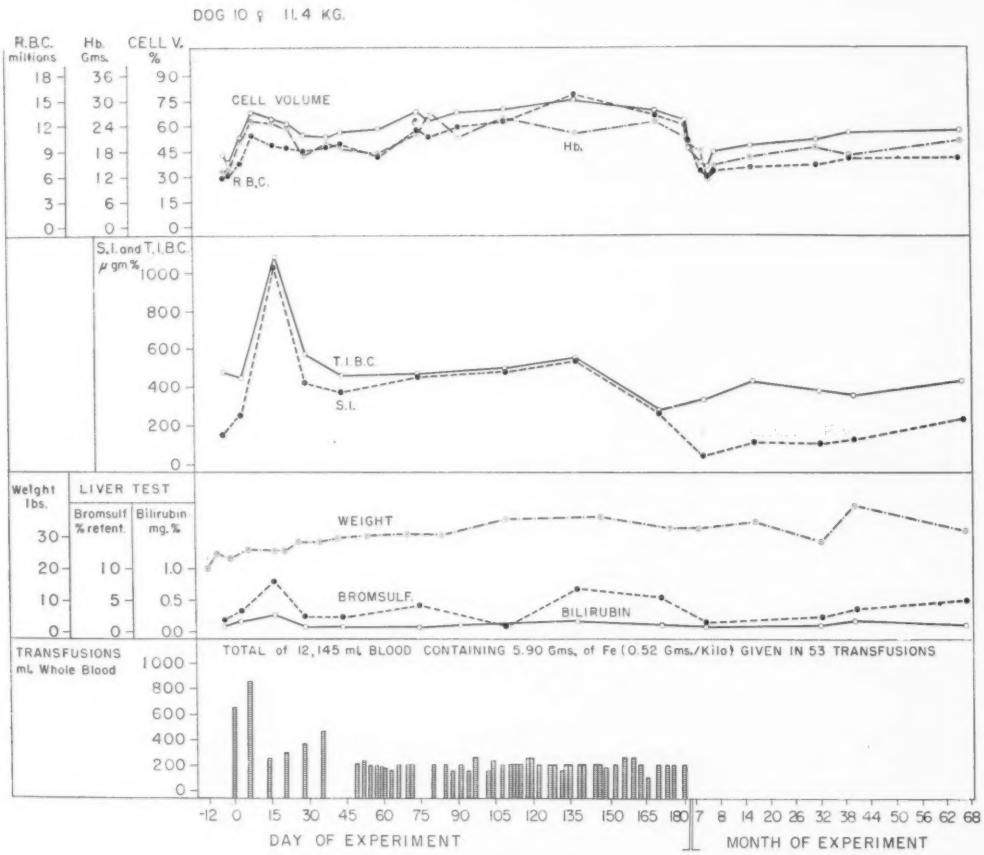


Fig. 2.—Effect of iron overload from multiple transfusions on the blood counts, serum iron, body weight, and liver function of a dog observed 6 years. The amounts of blood transfused and the total iron content are shown at the bottom of the chart.

noted. Enumeration of leukocytes, reticulocytes, and platelets was not done until after the completion of the period of intravenous iron injections. Intermittent eosinophilia as high as 20 per cent was observed in most animals and was thought to be related to intestinal parasitism. No other significant changes were obtained on differential leukocyte counts. Reticulocyte and platelet counts taken at infrequent intervals were normal throughout the period of observation.

Serum Iron Response.—Within a week after injections of saccharated iron oxide were begun serum iron values rose to between 400 and 1,000 μg per cent

with approximately 80 per cent saturation.* A plateau was then maintained until the seventh week of injections when a further rise to peak values usually greater than 2,000 μg per cent with 97 to 100 per cent saturation was seen. Except for Dog 18, discontinuation of intravenous iron injections resulted in a prompt fall in serum iron, though per cent saturation remained almost 100 per cent in all animals. Normal serum iron and per cent saturation levels were reached about 6 months after injections were completed, and except for consistently subnormal values for both measurements at 33 months, erratic variations within the normal range occurred thereafter.

Of the tranfused animals, Dog 10 behaved more nearly like those injected with Ferrivenin. The initial marked rise in serum iron and per cent saturation was related to the early massive amounts of blood this animal received. However, when blood was given in smaller quantities at a slower rate, both transfused dogs had only moderate rises in serum iron though the iron binding capacity remained saturated for prolonged periods. Except for its last measured serum iron, Dog 10 maintained low normal values from the eighth month onward. Serum iron and per cent saturation reached a low ebb in Dog 20 at 30 months and subsequently fluctuated within the normal range until the final low serum iron value at sacrifice.

Acute changes in serum iron values were measured at intervals for 24 hours after the intravenous injection of 100 mg. of Ferrivenin into 3 dogs. Two of these animals had received iron injections for a month, though no iron had been given for two days before measurement of acute serum iron changes; the third dog had received no previous iron injections. Prompt rise to extremely high serum iron levels (3,000 to 7,500 μg per cent) with complete saturation of the transferrin occurred uniformly at 30 minutes, followed by a rapid fall at 2 hours and a return to approximately base-line values after 24 hours.

Certain aspects of the serum iron changes in these dogs are worthy of emphasis. First, there were no evidences of toxicity despite transient elevations of serum iron above 7,000 μg per cent and sustained elevations greater than 2,000 μg per cent. The upper limits of iron binding capacity in the dog are normally less than 500 μg per cent. Since even small amounts of unbound ionic iron produce marked toxic reactions,[61, 62] the extremely high levels observed without incident suggest (1) circulation of undissociated saccharated iron oxide, (2) marked elevation of the beta-1 globulin binding capacity, or (3) binding by other serum proteins. There is no evidence bearing directly on the latter two possibilities but presence of unsaturated iron binding capacity when serum iron levels were greater than 2,000 μg per cent strongly suggests circulation of undissociated saccharated iron oxide. Even more difficult to explain is the serum iron value of 1,032 μg per cent and 96 per cent saturation observed at 3 weeks in one of the transfused dogs (10). Unless contamination of the sample is postulated, one must assume circulation of ferritin or some hemoglobin breakdown product,

*Per cent saturation is the quotient of serum iron and total iron binding capacity $\left(\frac{\text{SI}}{\text{TIBC}}\right)$. The total iron binding capacity is defined here as the sum of serum iron and unsaturated iron binding capacity. See following discussion for possible explanations why the iron binding capacity is not saturated despite such high serum iron values.

since the serum iron method does not measure iron present as hemoglobin. The plateau in serum iron after the initial high values from iron administration suggests the development of an increased clearing capacity that was later overwhelmed by the excessive iron load. After injections were discontinued, gradual clearance was achieved; overcompensation by clearing elements might have been present to account for the low normal values seen at 6 months. Somewhat surprising is the almost invariable desaturation of transferrin in the later periods of study despite the enormous tissue iron stores. Compared to the situation in human hemochromatosis or transfusion hemosiderosis where complete saturation is the rule, the storage iron in the dogs' tissues appears to be bound more tightly and not in as free exchange with the serum iron compartment.

Radioiron Studies.—The absorption of oral doses of radioactive iron was measured in 3 dogs with large tissue iron stores. Dog 18 was studied twice: initially, 3 months after completion of its course of Ferrivenin injections, and again 6 years later. Similar measurements were made once on Dog 6 about 6½ years after Ferrivenin injections, and on Dog 20 approximately 5½ years after completion of the series of transfusions. These values are tabulated (Table II).

TABLE II. ABSORPTION OF ORAL DOSE OF 1 MG./KG. OF RADIOIRON BY DOGS WITH IRON OVERLOAD

DOGS*	MONTHS AFTER LAST Fe ADMINISTRATION	PER CENT OF ORAL DOSE IN BLOOD	PER CENT OF ORAL DOSE IN FECES	PER CENT RECOVERY
18	3	1.7	98.2	99.9
	75	0.7	93.2	93.9
6	79	0.8	101.1	101.9
20	77	1.2	97.4	98.6

*Dogs 18 and 6 had received 1 Gm./Kg. of iron as Ferrivenin; Dog 20 had received 0.5 Gm./Kg. of iron from multiple transfusions.

Values for the absorption of comparable doses of orally administered radioactive iron in normal dogs vary from 0.8 to 8.8 per cent (average 5.8 per cent).[63] It appears from the figures that in these dogs with massive tissue iron deposits there was a low rate of iron absorption, but iron that was absorbed was quickly utilized for production of circulating hemoglobin.

Utilization for hemoglobin synthesis of intravenously administered radioactive iron (2.4 to 7.4 mg.) was measured in 3 dogs which had completed courses of saccharated iron oxide. Dogs 15 and 18 showed radioiron utilization values of 30 and 31 per cent, well within the normal range.[59] Dog 17, however, showed a greatly depressed iron utilization curve correlated most likely with the coincidental appearance of distemper. Similar depression of iron utilization has been reported in dogs and human beings during febrile illnesses.[59, 64, 65] If the curve for the dog with distemper is disregarded, the results of iron utilization studies on the other two animals suggest that despite the increased amounts of tissue iron, utilization of additional small amounts of intravenous iron was still within normal limits.

Tests of Hepatic Function.—Periodic evaluation of hepatic function was made by means of measurement of Bromsulphalein retention and serum bilirubin

TABLE III. TOTAL AND FRACTIONAL SERUM PROTEIN ANALYSES ON DOGS WITH IRON OVERLOAD

MONTHS AFTER START OF EXPERIMENT	DOG 6			DOG 20*			DOG 18			DOG 10			DOG 15			DOG 17		
	TOTAL PRO-TEIN	ALB.	GLOB.	TOTAL PRO-TEIN	ALB.	GLOB.	TOTAL PRO-TEIN	ALB.	GLOB.	TOTAL PRO-TEIN	ALB.	GLOB.	TOTAL PRO-TEIN	ALB.	GLOB.	TOTAL PRO-TEIN	ALB.	GLOB.
17	6.0	4.6	1.4	7.0	3.5	3.5	7.0	4.8	2.2	5.6	4.0	1.6	6.5	4.2	2.3	7.0	4.9	2.1
33	6.4	4.8	1.6	6.9	3.6	3.3	6.0	4.4	1.6	6.1	4.4	1.7	6.5	4.7	1.8	6.5	5.0	1.5
40	6.0	3.7	2.3	6.7	3.8	2.9	5.3	3.5	1.8	6.0	3.8	2.2	5.8	3.9	1.9	5.2	3.8	1.4
67	6.0	3.7	2.3	5.9	3.9	2.0	5.0	3.2	1.8	5.7	3.9	1.8	5.9	4.8	1.1	--	--	--
74	5.4	2.7	2.7	6.2	4.5	1.7	5.0	2.4	2.6	--	--	--	--	--	--	--	--	--
79	6.3	3.4	2.9	6.1	--	--	4.9	2.7	2.2	--	--	--	--	--	--	--	--	--
81	5.9	3.4	2.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
84	--	--	--	6.2	1.8	4.4	4.7	2.8	1.9	--	--	--	--	--	--	--	--	--
85	--	--	--	5.9	1.6	4.3	--	--	--	--	--	--	--	--	--	--	--	--

*The experiment on Dog 20 was begun 3 months after those on the other animals. Hence, the time of each sample is 3 months less than that shown in column one.

Dogs 6 and 18 had received iron as Ferrivenin in doses of 1.0 Gm./Kg.; Dogs 15 and 17 had received 0.5 Gm./Kg. of Ferrivenin; and Dogs 10 and 20 had received 0.5 Gm./Kg. of iron from multiple transfusions.

levels. To these tests serum protein determinations were added 17 months after the start of iron administration. Paper electrophoretic analyses of serum protein patterns were also determined during the last 18 months of the experiment.

All dogs had normal Bromsulphalein retention (less than 12 per cent at 30 minutes)[66] except Dog 17 which had 22.4 per cent retention before any iron had been given, and Dog 18 which showed an isolated elevation of 13.7 per cent 5 weeks after the start of iron injections. No pattern of even minor impairment of Bromsulphalein clearance could be discerned, nor were any differences noted between the animals given Ferrivenin and those transfused. Likewise, measurement of serum bilirubin failed to reveal abnormalities in any animal. The indirect reacting fraction initially tended to be higher in the transfused animals in which large amounts of hemoglobin were being catabolized, but in no case were total bilirubin values greater than 0.5 mg./100 ml. observed. The most striking changes in serum proteins occurred in Dog 20 which showed a gradual fall in total proteins, a marked terminal fall in albumin and a rise in globulins with reversal of the albumin-globulin ratio (Table III). Dog 18 likewise showed a progressive fall in total proteins due primarily to a fall in albumin but with no reversal of albumin-globulin ratio. Dog 17 likewise showed a progressive fall in total proteins in the three determinations performed. No conclusive patterns were discernible in the remaining animals. Analysis of paper electrophoretic separations of serum protein components confirmed the marked changes described above, with the globulins increased mainly in the gamma fraction. Less marked variations from normal were seen in the patterns of the other dogs.

With the exception of the serum protein changes, all dogs showed no evidence of abnormal liver function whether they received saccharated iron oxide or transfusions. The reversal of albumin-globulin ratio demonstrated by Dog 20 is the only change suggestive of cirrhosis; the protein changes in the other animals are nonspecific. Whether Dog 20's deviation from normal is related to the multiple transfusions or to hepatic iron storage cannot be answered unequivocally.

Tests of Glucose Tolerance.—Abnormal glucose tolerance associated with iron overload of the pancreas and/or the liver was evaluated by means of repeated fasting blood sugar determinations on all dogs and by oral glucose tolerance tests in 3 animals. No elevation of fasting blood sugar was obtained on any dog throughout the experiment. If any abnormality could be found, it was a mild hypoglycemic tendency. Each animal showed blood sugar levels of less than 50 mg./100 ml. at some time during its course. Patterns of blood sugar values were similar in dogs injected with Ferrivenin and those which were transfused.

Approximately 80 months after the start of iron administration, oral glucose tolerance tests were done on Dogs 6, 18, and 20; the results are shown graphically (Fig. 3). Compared with normal dogs (shaded area), these animals showed no significant deviation except for Dog 20. In this dog, the same one which showed albumin-globulin reversal, there was a moderate but sustained elevation of blood sugar throughout the 3½ year period of study. While this curve reveals diminished glucose tolerance, it is not diagnostic of diabetes.

Thus, with one possible exception, prolonged iron overload from transfusions or injected saccharated iron oxide failed to produce any laboratory evidence of diabetes mellitus.

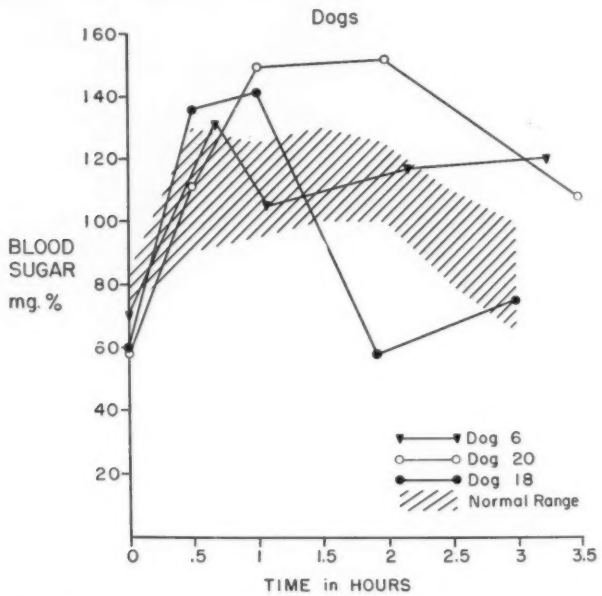


Fig. 3.—Oral glucose tolerance curves of three dogs with iron overload. Dogs 6 and 18 had received 1.0 Gm. per Kg. of iron as saccharated iron oxide, and Dog 20 had received 0.5 Gm. per Kg. of iron from multiple transfusions. The dose of glucose was 3 Gm. per Kg. of a 20 per cent solution. The normal range of glucose tolerance is shown in the shaded area.

Miscellaneous Observations.—Electrocardiograms were taken on Dogs 6, and 18 both 8 and 79 months after the beginning of Ferrivenin injections, and a single tracing was taken on Dog 20 at 76 months after the start of transfusions. The tracings were interpreted as being within normal limits.

The insidious development of blindness was observed in all animals beginning about 3 years after the start of iron administration and proceeding to complete loss of light perception. Ophthalmoscopic examination shortly before sacrifice revealed multiple small opacifications in the vitreous, perivascular sheathing of retinal vessels and degenerative changes of retinal nerve cell layers most marked at the periphery. These changes suggested that iron accumulation in the eye produced a retinopathy similar to siderosis bulbi and retinitis pigmentosa.

Two pups were born to Dog 6 approximately two years after the mother had received the 1 Gm./Kg. dose of saccharated iron oxide. These pups appeared to be normal, healthy animals and grew satisfactorily. At the time of sacrifice at 34 days of age both pups showed a mild anemia, and the large male pup had a serum iron level below normal. Despite the massive tissue iron concentration in the mother, there was no increase in the iron concentration of the pups' tissues. Histologic sections of selected tissues disclosed no abnormalities.

Tissue Iron Determinations.—Measurements of iron concentrations of tissues that could be readily biopsied were made on several occasions in order to quantitate the degree of iron accumulation and delineate any redistribution of iron that might occur. At autopsy, iron estimations of the biopsied organs and of additional tissues not accessible to biopsy were made. Estimates of the iron content of the major anatomical divisions of the body were calculated and by their summation an attempt was made to account for the iron administered parenterally.

All four dogs which received saccharated iron oxide underwent laparotomies before the start of iron injections, and the iron content of liver biopsies was determined. Likewise iron analyses of liver samples were performed initially on Dogs 12 and 13 which died early in the course of transfusions. No pretransfusion liver iron determinations were made on Dogs 10 and 20 which ultimately survived the complete course of transfusions. However, in all dogs biopsied, the initial iron concentration of the liver ranged from 0.081 to 0.343 mg./Gm. of wet tissue with an average of 0.205 mg./Gm. These figures agree well with those given for normal dogs by Bogniard and Whipple.[67]

Skin biopsies for iron analysis were obtained from the inner aspect of the thighs of all dogs which received Ferrivenin one to four weeks after their course of injections had been completed. These biopsies contained iron in concentrations varying from 0.160 to 0.386 mg./Gm.; the normal average is 0.025 mg./Gm.

Laparotomies were again performed 6 months after beginning iron injections on each of the 4 dogs which had received Ferrivenin, and for the first time on Dog 10 which had just completed its course of transfusions. Tissue iron estimations were made on biopsies of liver, spleen, pancreas, kidney, muscle, skin, and mesenteric lymph node. These values and those of later biopsies are shown in Table IV.

Three years after the start of transfusions or Ferrivenin injections biopsies were repeated and samples of liver and spleen were analyzed for iron as before. No discernible pattern was evident in the changes of iron concentration of liver and spleen specimens from these repeated post-iron biopsies. One animal, Dog 6, had consistently higher concentrations of iron in the liver than in the spleen; Dogs 18 and 10 showed the opposite pattern repeatedly; and Dogs 15 and 17 showed reversals of maximal iron concentrations in liver and spleen on successive determinations. Despite the difference noted, it is clear that iron given to these dogs accumulated in high concentrations in both spleen and liver reaching values as much as 50 to 75 times normal.

The concentration of iron in various tissues sampled at autopsy is given in Table V. Notable is the remarkable uniformity of iron values obtained from heart, brain, pancreas, and stomach in all dogs with or without excessive iron stores, emphasizing the absence of accumulation of iron in these organs. The moderate variations in iron concentration of lungs, adrenals, and mesenteric nodes and the marked fluctuations in liver, spleen, bone marrow, and peripancreatic lymph nodes undoubtedly reflect true differences in iron deposition rather than sampling errors. These figures emphasize the predominant localization of iron in organs with large reticuloendothelial components.

TABLE IV. IRON CONCENTRATION (MG./GM. WET WEIGHT) OF TISSUES OBTAINED AT BIOPSY OF DOGS WITH IRON OVERLOAD

TIME OF BIOPSY	DOG 6		DOG 18		DOG 20		DOG 10		DOG 15		DOG 17	
	LIVER	SPLEEN	LIVER	SPLEEN	LIVER	SPLEEN	LIVER	SPLEEN	LIVER	SPLEEN	LIVER	SPLEEN
Pre-iron control	0.81	---	0.343	---	---	---	---	---	0.222	---	0.223	---
Four months after iron	9.07	3.07	15.1	16.05	---	---	5.82	11.6	7.50	3.72	1.68	3.86
36 months after iron	14.8	6.98	8.04	13.0	10.1	12.45	4.3	4.86	5.55	11.4	6.82	4.14

TABLE V. IRON CONCENTRATION (MG./GM. WET WEIGHT) OF TISSUES OBTAINED AT AUTOPSY OF NORMAL DOGS AND DOGS WITH IRON OVERLOAD

TISSUES	DOG 6	DOG 18	DOG 20	DOG 10	DOG 15	DOG 17	NORMAL DOG (AVERAGE)
Liver	7.08	4.98	4.26	2.11	2.94	5.00	0.20
Spleen	5.23	6.90	14.46	4.28	3.58	11.6	0.24
Pancreas	0.05	0.04	0.05	0.11	0.07	0.13	0.06
Kidney	0.23	0.13	0.14	0.19	0.15	0.15	0.08
Heart	0.04	0.05	0.06	0.09	0.07	0.07	0.07
Lungs	0.22	0.14	0.20	0.36	0.36	0.26	0.25
Stomach	0.06	0.04	0.04	---	---	---	0.04
Mesenteric node	0.35	0.16	0.27	0.36	0.24	---	0.06
Peripancreatic node	18.65	11.8	5.34	---	---	3.36	---
Vertebral marrow	2.22	8.53	1.63	0.66	0.66	1.77	---
Femoral marrow	4.04	2.46	0.98	---	---	---	0.22
Muscle	0.04	0.27	0.05	0.03	0.03	0.06	0.04
Abdominal skin	0.03	0.14	0.03	0.29	0.09	0.01	0.03
Brain	0.02	---	0.01	0.03	0.08	0.07	0.02
Eye	0.27	0.02	0.02	0.02	0.18	---	0.01
Thyroid	0.21	0.05	0.05	---	---	---	0.06
Adrenal	0.30	0.23	0.13	---	---	---	0.07

An attempt was made to estimate total carcass iron from the product of the organ concentrations and weight plus estimates of skin, muscle, and bone marrow iron increments. To the carcass iron estimates were added the iron loss from repeated blood sampling, and in Dog 6 the iron loss due to the birth of pups. The sum of this accountable iron was compared to the amounts of iron given each dog. As seen in Fig. 4, 37 to 73 per cent of the injected iron could be accounted for by such a calculation. Though greatest recovery was found in the two dogs receiving the largest concentrations of iron as Ferrivenin, recovery of iron seemed independent of length of survival or type of iron given.

Some of the reasons for failure of complete recovery of administered iron can be enumerated. (1) There may be considerable error in estimating the iron content of muscle, skin, lymph nodes, and bone marrow since these organs cannot be easily weighed. (2) Sampling errors, despite duplicate measurements, in tissues with high iron content such as liver, spleen, and bone marrow are magnified in total organ iron estimates. (3) An unknown, but possible appreciable loss of iron may have occurred via the gastrointestinal tract due to blood loss from intestinal parasites. Despite frequent tests for occult blood in the feces and repeated use of vermifuges, parasites were known to be present and were found at autopsy in two dogs. Loss of as little as 1 ml. of blood daily by this means would account for as much as 1.25 Gm. of iron during a 7-year period. (4) Loss of saccharated iron oxide in the urine during the period of tremendously elevated plasma iron levels might further account for considerable iron loss. Measurement of radioiron excretion acutely after intravenous injection of 100 mg. doses of Fe^{59}-tagged Ferrivenin revealed approximately 1 per cent excretion in a normal dog and about 5 per cent excretion in a dog with massive iron overload. If one assumes an intermediate figure of 2 per cent for these animals an additional 100 to 200 mg. of iron might have been

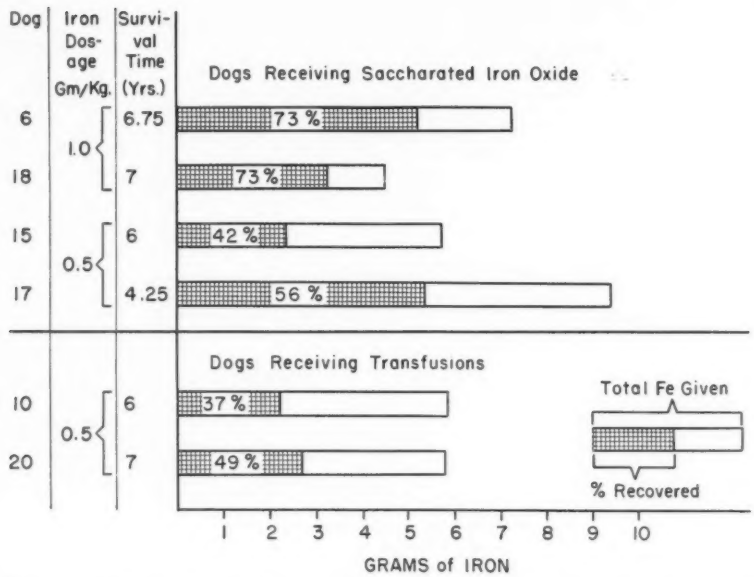


Fig. 4.—Estimate of the iron recovered at autopsy from the tissues of 6 dogs with iron overload, plotted as per cent of the total iron injected. The total amount of iron given to each dog is indicated by the scale at the bottom of the chart.

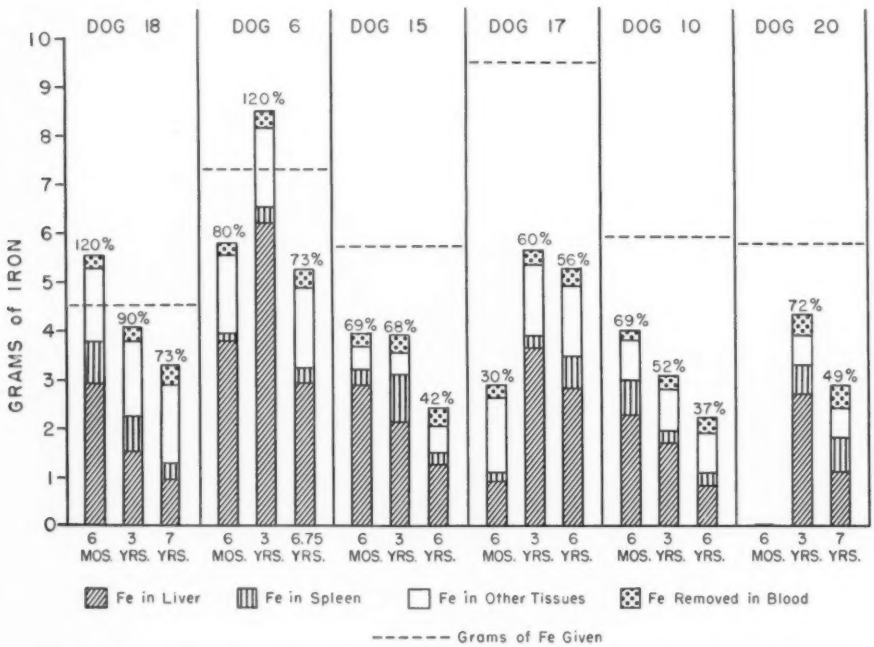


Fig. 5.—Shifts in body iron observed in 6 dogs with iron overload, estimated from the iron found in successive biopsy specimens and in the tissues at autopsy. The time of the biopsy or the autopsy is given at the bottom of each column. In each case, the column farthest to the right represents the autopsy data.

lost by this route. (5) Finally, increased excretion of iron through normal channels by these dogs with massive iron stores may have occurred. Such a phenomenon has been reported in iron-heavy mice by Finch and associates.[68, 69] Since radioiron excretion studies were not performed on these animals, no answer can be given to this question. However, to account for the iron deficits noted by assuming increased excretion alone, one would have to postulate daily iron excretion of 0.5 to 2.6 mg. Excretion values corresponding to the lower figure have been reported for dogs with phenylhydrazine-induced hemolytic anemia,[70] but a level of iron excretion high enough to explain the discrepancy between the iron administered and the iron recovered seems improbable.

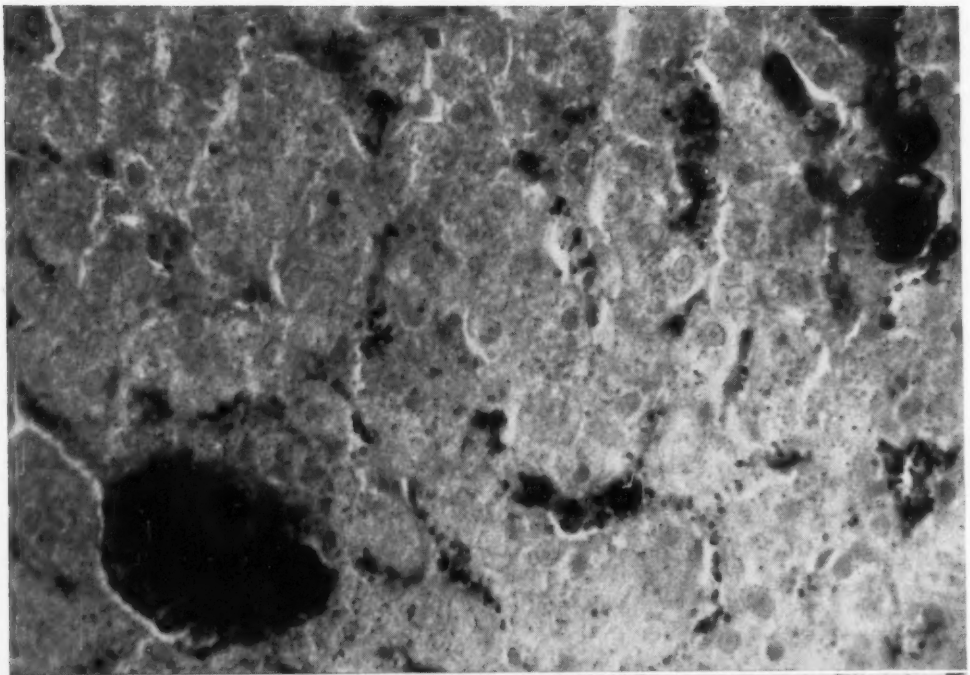
Furthermore, if a constant increased rate of iron excretion occurred it should be possible to detect it by comparing estimations of the total carcass iron at the time of the 6 and 36 month biopsies with that at autopsy. In order to make this comparison, certain assumptions are necessary; namely, (1) the weights of various organs remained constant throughout the experiment and were the same as at autopsy, (2) the iron content of organs other than liver and spleen remained the same as found at autopsy and (3) correction was made for iron withdrawn by blood sampling at the various times. Then the iron content of liver and spleen was calculated from the weights of these organs multiplied by their iron concentrations at biopsy or autopsy. To these figures were added the iron content of other tissues and the increment due to blood sampling, and this total was compared to the amount of iron given during the course of the experiment. The results of such an analysis are presented in Fig. 5. In Dogs 18, 15, 10, and 20 a progressive decline in liver iron was observed paralleled by a fall in estimated total carcass iron recovery. Iron content of the spleen decreased progressively in Dogs 18 and 10, increased in Dogs 17 and 20, and oscillated in Dogs 6 and 15. By these admittedly crude calculations, total body iron estimated from biopsy values exceeded the amounts given by 20 per cent on two occasions. Thus, while recovery of iron at autopsy in all but one dog was lower than the estimated recovery earlier in the experiment, the evidence for a constant increased excretory loss is meager.

Histologic Observations.—Throughout the course of the study, an effort was made to evaluate the sites of deposition of iron given parenterally and to delineate the histologic pattern of reaction to the increased tissue iron by repeated biopsies of accessible tissues and by extensive sampling at autopsy.

Control biopsies: The initial histologic sections of the dogs' tissues obtained at laparotomy before administration of iron revealed no abnormalities in the liver, spleen, pancreas, mesenteric lymph node, skeletal muscle, and skin. In none of the dogs was there any evidence of fibrosis or cirrhosis either by hematoxylin and eosin or connective tissue stains. Iron stains of these various tissue sections revealed a small amount of iron in the macrophages of the spleen and liver and rarely in the macrophages about the central sinusoids of the mesenteric lymph nodes. No evidence of hemofuscin was found in any section.

Post-iron biopsies: Approximately one month after completion of Ferrivenin injections, skin biopsies were obtained on each of the 4 dogs. Iron deposition confined to the upper layers of the dermis, especially around the hair follicles, was the only noteworthy finding.

Fig. 6.



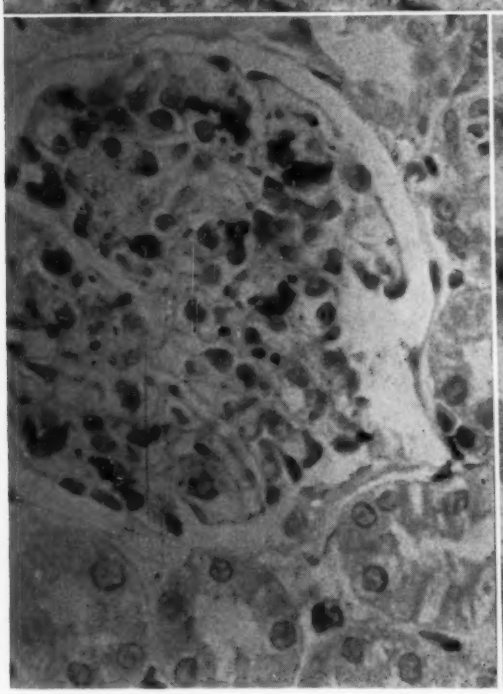


Fig. 7.

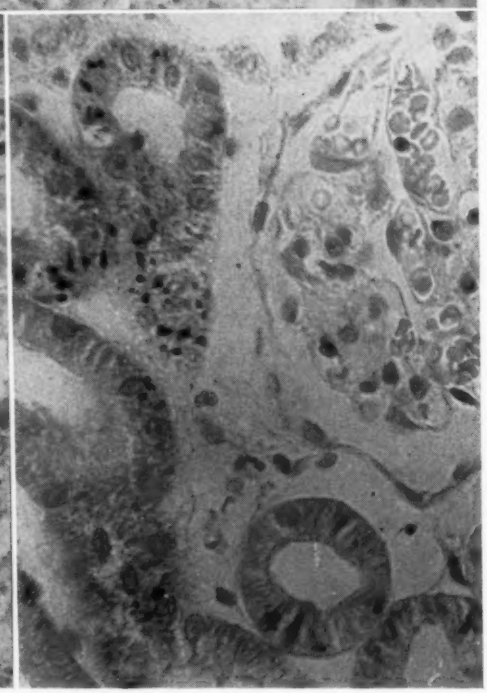


Fig. 8.

Fig. 6.—Liver of Dog 10 one month after completion of transfusions. There are large aggregates of granules that react positively for iron (black in photograph) in clumps of macrophages in the sinusoids and finer deposits along the sinusoidal endothelium and within the hepatic cells. There is no fibrosis or hemofuscin. Gomori's stain. (Magnification ×595.)

Fig. 7.—Kidney of Dog 6 four months after 1.0 Gm. per Kg. of Ferrivenin. Iron positive granules are present in endothelial cells of capillaries and in macrophages caught in the glomerular tuft. Gomori's stain. (Magnification ×595.)

Fig. 8.—Kidney of Dog 10 one month after transfusions. Iron positive granules are present in tubular epithelium but not in the glomeruli. Gomori's stain. (Magnification ×595.)

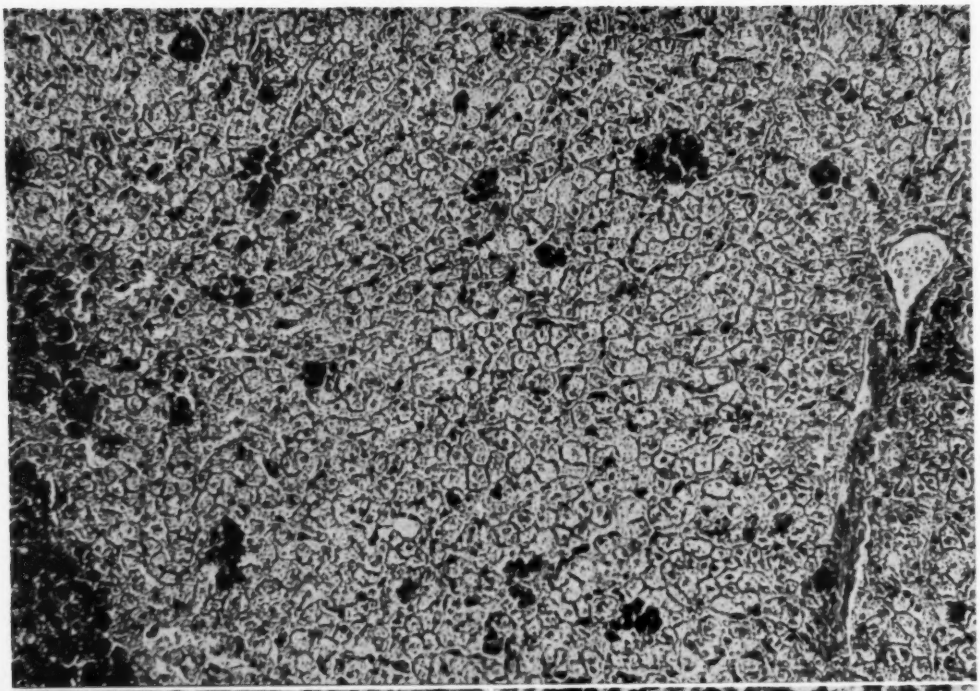
A second laparotomy was done 4 months after Ferrivenin injections were completed (Dogs 6, 18, 15, 17) and additional biopsy specimens for histologic examination were obtained. In Dog 10 a laparotomy with biopsy of the aforementioned organs was performed approximately one month after completion of transfusions. Except for the sections of the kidney, the histologic appearance of the various organs was identical for the various dogs and was independent of the dose of iron whether administered as Ferrivenin or transfused blood. Sections stained with hematoxylin (H) and eosin (E) revealed marked pigmentation of the liver, spleen, and mesenteric lymph nodes; considerably less pigment in pancreas, kidney, and skin; and practically no pigment in the skeletal muscle. There was no evidence of increased fibrous tissue in the liver, pancreas, or other organs when stained with either H and E or Masson's stains, and there were absolutely no cirrhotic changes. Iron stains of the liver revealed hemosiderin to be deposited in coarse granules aggregated into clumps with focal distention of sinusoids and deposits in the portal triads. In addition to these larger deposits there was a diffuse, fine, granular deposition of iron in the hepatic parenchymal cells distributed uniformly throughout the lobule and in individual Kupffer cells (Fig. 6). Smaller amounts of iron were seen in the endothelium of the blood vessels, but no iron was found in the bile ducts. In the spleen, the iron was found in largest quantities in the macrophages of the sinusoids and to a lesser extent in the capsule, trabeculae, and beneath the endothelium of the blood vessels. No iron was seen in Malpighian corpuscles. Iron in the kidneys of the dogs given Ferrivenin (Fig. 7) was confined to a moderate amount in the glomerular capillary tufts with only a faint blue shade and rare, fine granules in the renal tubules. In contrast, the transfused dog showed small amounts of iron as fine blue granules in the proximal convoluted tubules with practically no iron seen in the glomeruli (Fig. 8). The pancreas in both groups revealed only small amounts of iron confined to macrophages and the endothelial layers of the blood vessels. There was no iron deposition in the acinous or islet epithelial cells, and no evidence of other pigment suggestive of hemofuscin. Mesenteric lymph nodes showed large quantities of iron in the macrophages of the central sinusoids with a less marked pigmentation of the peripheral macrophages. Iron in the skin was confined to macrophages in the dermis, especially those around hair follicles, and was increased in amount compared to that seen in the skin biopsy sections taken 3 months previously. There was no iron or other pigmentation of the epidermis. The only iron seen in skeletal muscle sections was a faint blue cast to the capillary endothelium in one dog. No hemofuscin was seen in any of the sections.

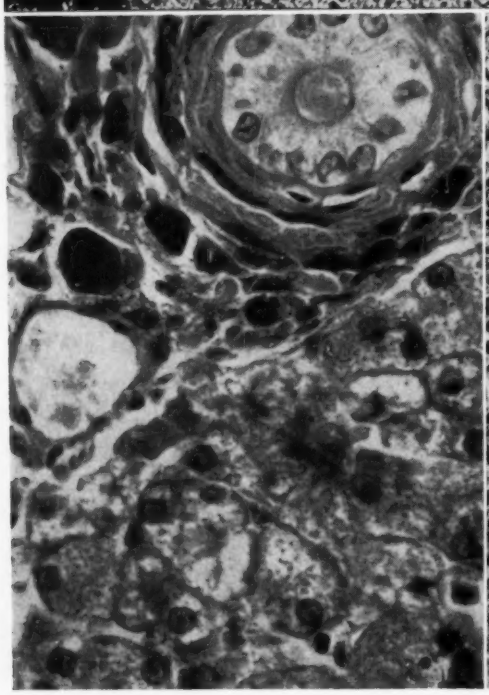
Laparotomies, with biopsies of abdominal organs previously described, were performed on all 6 dogs approximately 3 years after completing iron administration. Histologic appearance of the tissues was identical to that seen at autopsy and a single description will suffice.

Autopsies: Gross examination revealed no free fluid in the pleural, pericardial, or peritoneal cavities of any of the dogs. Fibrous adhesions of the peritoneum were seen in most dogs at sites of old anterior abdominal incisions and organ biopsies. A distinct, rust-brown color was noted in the lymph nodes, pancreas, spleen, and kidney; the liver was a dark mahogany-brown. In the two dogs with post-mortem autolysis, a diffuse, shiny, black pigmentation, presumably due to iron sulfide, was present in all abdominal organs and with greatest intensity in the intestines. In two dogs Ascaris infestation was demonstrated in the upper portion of the small intestine. A single laminated gallstone was found in the gall bladder of Dog 6. No abnormalities of heart, lungs, great vessels, brain, or other organs were apparent grossly.

Microscopic appearance of the tissues of all dogs is described in composite fashion except in those cases where distinctive changes were present. The basic architecture of the liver was normal (Fig. 9) with no evidence of cirrhosis or even increased fibrous tissue in any animal. The iron was present principally in large compact clumps of heavily laden macrophages (siderophages) which were present at random sites in the sinusoids and in large masses in the portal triads. Individual Kupffer cells also contained moderate amounts of iron. There was only the faintest hint of fine hemosiderin granules in the liver parenchymal

Fig. 9.





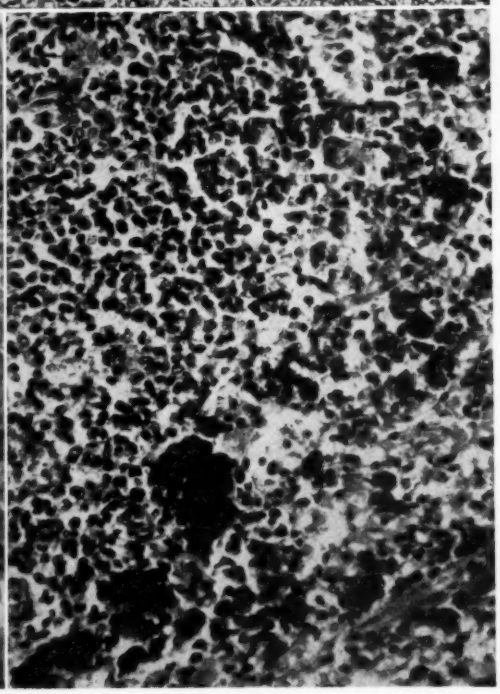


Fig. 10.

Fig. 11.

Fig. 9.—Liver of Dog 6 at autopsy 84 months after 1.0 Gm. per Kg. of Ferrivenin. Iron is present almost entirely within clumps of macrophages in the sinusoids and portal spaces. There is no fibrosis or disturbance of lobular architecture. Hematoxylin and eosin. (Magnification ×290.)

Fig. 10.—Liver of Dog 6 at autopsy (same section as Fig. 9). There are no iron granules in the hepatic cells or epithelium of the bile ducts. Hematoxylin and eosin. (Magnification ×595.)

Fig. 11.—Spleen of Dog 6 at autopsy. Clumps of macrophages filled with iron occur throughout the red pulp but especially at the perimeters of the follicles. Hematoxylin and eosin. (Magnification ×290.)

cells and no evidence of iron in the bile ducts (Fig. 10). The principal change from the earlier biopsies consisted of a more compact concentration of reticuloendothelial (R.E.) elements loaded with iron and a fading of what slight evidence there had been previously of iron in the epithelial tissue. No hemofuscin was seen in liver sections of any dog.

Sections of spleens (Fig. 11) showed huge clusters of iron-laden macrophages plus scattered single macrophages throughout the red pulp with no such cells in the follicles. There was a thick reticulum about the sinusoids of the red pulp. Dog 20 showed scattered megakaryocytes in the red pulp.

Lymph nodes were similar to those previously described at biopsy, showing heavy concentrations of siderophages in the central and peripheral sinusoids and cords of lymphoid tissue but not in the follicles. Some nodes showed siderophages in the capsular connective tissue. The amount of iron varied from node to node, though highest concentrations were uniformly seen in peripancreatic nodes. There was no evidence of fibrosis in any node examined.

No fibrosis, atrophy, or iron deposition in epithelium of ducts, islets or acini of the pancreas was found (Fig. 12). An occasional iron-filled macrophage was present at random sites in the intralobular connective tissue.

The kidneys were of basically normal appearance. Iron granules in the glomerular capillary tufts in the biopsy sections of dogs injected with Ferrivenin had entirely disappeared. The only remaining iron was seen in an occasional interstitial R.E. cell in the cortex or as a faint blue granular staining of occasional cells in the proximal convoluted tubules. More frequently tubular epithelium contained a brown noniron pigment that was probably some form of urochrome. Calcification of tubules in a small area of medulla was noted in 2 dogs with moderate numbers of siderophages associated with a slight inflammatory reaction in the area. No nephritic lesions of the glomeruli were seen. The urinary bladder of Dog 6 showed chronic cystitis in which siderophages had accumulated in association with the lymphocytic infiltrate in the lamina propria. In Dog 18 there was the barest evidence of the residue of a similar process with scattered siderophages in the lamina propria.

No abnormalities, except for incidental fatty infiltration of the myocardium in Dog 20, were observed in the hearts of these dogs. No stainable iron was seen. In the lungs iron-containing macrophages were seen in the alveolar walls. The location of these macrophages suggested that they were R.E. cells that had been trapped in the alveolar capillaries rather than iron-laden macrophages in the alveolar lumens seen in chronic congestion or the scavenger cells which accumulate in lymphoid channels along bronchial trunks. Dog 6 showed an incidental calcified subpleural nodule; Dog 18 showed a heavy amount of anthracosis; and Dog 20 showed several focal granulomas of nonspecific etiology.

Bone marrow from ribs, vertebrae, and femur showed clumps of heavily laden siderophages scattered at random throughout the marrow. There appeared to be normal marrow cellularity in all animals, though Dog 6 seemed to have some reduction in erythroid elements. The bone was of normal appearance.

All sections of the brain and spinal cord were remarkable only for scattered siderophages in the pia-arachnoid and rarely beneath the ependyma or in the stroma of the choroid plexus. There was no evidence of iron deposition in neurons, ependymal cells, or epithelium of the choroid plexus.

Skeletal muscle showed no abnormalities and no iron in any of the sections studied. In the adrenals, thyroid, pituitary, and salivary glands there were no structural abnormalities. Iron was present in isolated macrophages in the interstitial tissues but none was seen in epithelial cells. In one dog, a noniron-containing fuchsinophilic, brown pigment was found in the epithelial cells of the thyroid.

The testes of Dog 20 were almost completely atrophied and fibrotic with persistent islands of interstitial cells. Iron-laden macrophages were present in looser areas of the scar. In the prostate of this animal there were foci of chronic interstitial inflammation within which were scattered siderophages. In the ovaries of Dogs 6 and 18 there were rather heavy masses of siderophages in foci in the stroma and in corpora albicantia but not in more recent follicles or corpora lutea. In the uterus of one dog there were siderophages scattered throughout the stroma of the endometrium.

Fig. 12. Fig. 13.

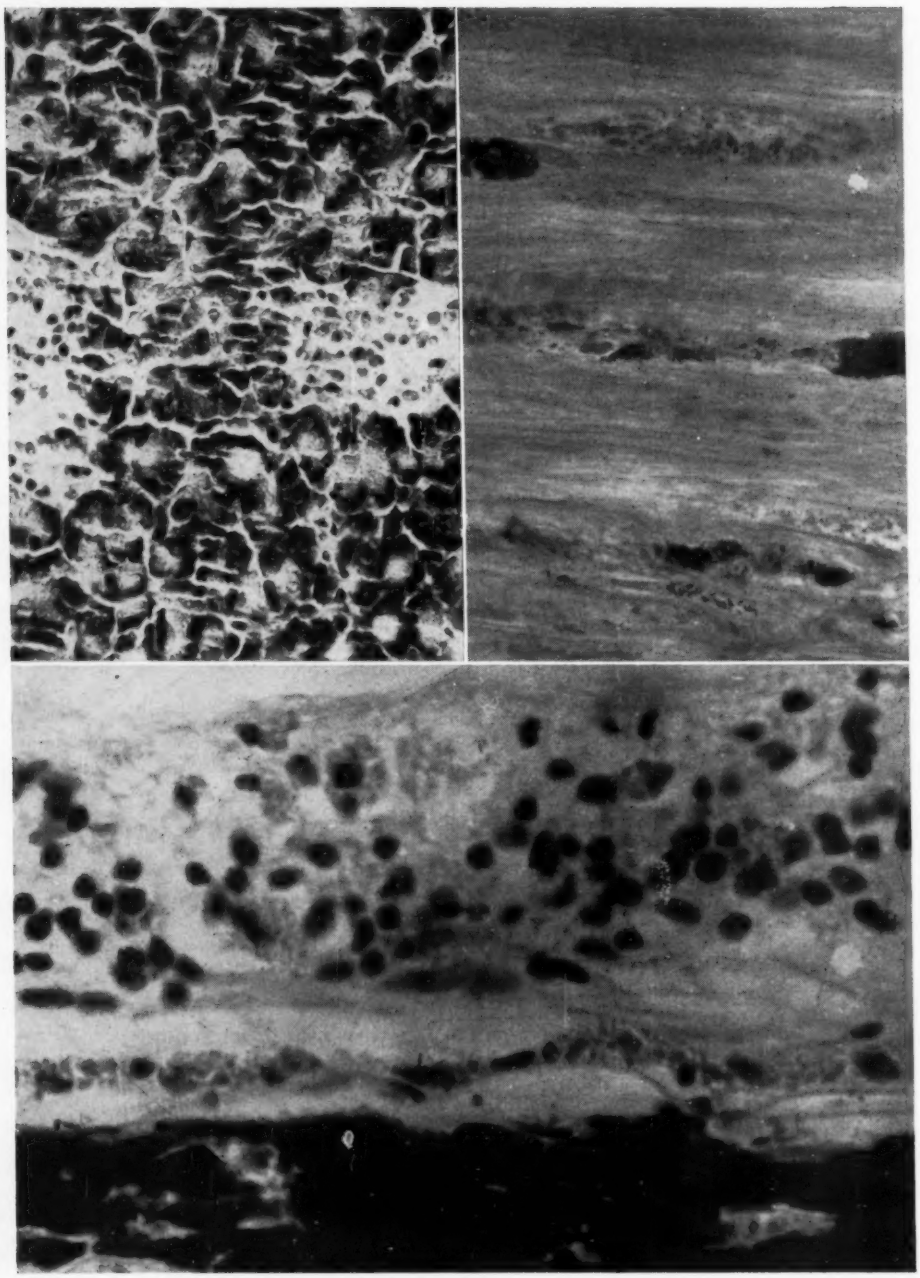


Fig. 14.

Fig. 12.—Pancreas of Dog 18 at autopsy 81 months after 1.0 Gm per Kg. Ferrivenin. There is no evidence of atrophy or fibrosis. Only occasional granules of iron are present in scattered interstitial macrophages. Hematoxylin and eosin. (Magnification ×290.)

Fig. 13.—Stomach of Dog 6 at autopsy. The coarse granular and fine refractile forms of the pigment with the staining reactions of hemofuscin are present in and between the cells of the muscularis. Turnbull blue stain with basic fuchsin counterstain. (Magnification ×595.)

Fig. 14.—The retina from Dog 18 at autopsy. There are characteristic changes of retinitis pigmentosa with many cells containing retinal pigment and a few with iron. Hematoxylin and eosin. (Magnification ×250.)

Breast tissue from two female dogs revealed siderophages in the interstitial tissues of the lobules and a few granules staining for iron in the epithelial cells, especially in obstructed, dilated, and atrophic ducts and lobules.

Sections of tongue and esophagus were not remarkable except for a few small nonspecific granulomas in the lamina propria and muscularis of the tongue in Dog 20. Throughout the remainder of the gastrointestinal tract the mucosa showed no pathologic changes except for very rare siderophages in the lamina propria. These were most numerous just above the muscularis mucosa in the upper intestine. A chronic gastritis represented by an unusually abundant lymphocytic and plasmacytic infiltration in the lamina propria was seen in Dog 20.

Beginning in the stomach and extending throughout the small intestine and colon, there was a moderate to heavy deposition of noniron-containing pigment in the muscularis. The intensity of this pigmentation was maximal in the upper small intestine; it was greatest in Dog 6 and least in Dog 20. What was apparently the same pigment was deposited in two forms (Fig. 13). The first was a golden brown, intense staining of the cytoplasm of mononuclear wandering cells that were present in small groups along and between the smooth muscle fibers. The second form was a glossy brown, finely granular and rather rarefied form that appeared in swollen and vacuolated muscle cells adjacent to the sites of interstitial deposition. This pigment showed no fluorescence; it stained heavily with basic fuchsin, methylene blue, and periodic acid-Schiff stains but did not stain with iron or acid-fast reactions except for occasional acid-fast granules, nor with Sudan IV on sections embedded in paraffin. Except for the presence of a similar pigment in the muscularis of the gall bladder in one dog and minute amounts in the blood vessels of pancreas and salivary glands, this pigment was recognized only in the smooth muscle of the gastrointestinal tract and the epithelium of the thyroid. Where deposits were particularly heavy there was an accompanying infiltrate of plasma cells and lymphocytes between the muscle fibers. The infiltrate seemed to be secondary because it was not present in sites of less intense pigmentation.

As mentioned above, the gall bladder of one dog (6) showed a glossy brown pigment in the cells of the muscularis similar to that described in the smooth muscle of the gastrointestinal tract. In all dogs the only evidence of iron was in a few macrophages in the lamina propria.

The eyes of all dogs, whether they received iron as saccharated iron oxide or as transfused hemoglobin, showed changes characteristic of retinitis pigmentosa (Fig. 14). These changes consisted of marked glial proliferation of the retinal nerve cell layers; degeneration of the choriocapillaris; migration of moblized retinal pigment epithelia into the retina, tapetum lucidum, and choroid; and very rare iron-containing macrophages. In one dog (20) lenticular opacification was also noted.

From the histologic findings just summarized certain features bear emphasis. First, the histologic appearance of all organs studied, with the single exception of the kidney was identical whether iron was given as hemoglobin or as saccharated iron oxide. Second, the distribution of iron was predominantly reticuloendothelial, with huge aggregates in liver, spleen, bone marrow, and lymph nodes and small amounts in scattered macrophages in interstitial tissues wherever R.E. elements might be present. Epithelial cell iron deposition was confined to small amounts in the liver, occasional renal tubular cells, and the breast. Third, increased iron deposition was found wherever there were sites of old inflammation or trauma such as cystitis, interstitial nephritis and in old ovarian follicles. Whether these sites of predilection were the result of the attraction of iron-laden wandering macrophages to sites of inflammation or whether iron from hemorrhage at these sites was picked up by R.E. cells but never moved away because of the saturation of usual depots of removal cannot be answered definitively. Fourth, no evidence of increased fibrosis or

cirrhosis could be found in any organ examined. Fifth, a pigment with staining characteristics most nearly like those of the lipochrome hemofuscin was found in smooth muscle layers of gastrointestinal tract, in one gall bladder and in the epithelium of the thyroid and salivary glands. The source and significance of this pigment are unknown. Finally, lesions in the eyes severe enough to produce blindness and pathologically similar to those of the end stages of retinitis pigmentosa were present in all dogs that survived to the end of the experiment. It is true that the causal relationship between iron deposition and the retinal changes is not proved, but additional unpublished evidence suggests it to be present. Prolonged tissue iron overload in these dogs was remarkably well tolerated and produced minimal histologic alterations.

DISCUSSION

Despite the use of large doses of iron and periods of follow-up longer than have ever previously been described, our attempts to produce hemochromatosis in dogs have failed just as have those of other investigators. Repeated failure in such attempts prompts a search for a possible explanation, or at least a reorientation of the facts so that future experiments may be redesigned for greater success.

One of the more plausible explanations for the failure to produce hemochromatosis in these and other animals is that there may be a species difference whereby man is unique in his reaction to iron, and a similar response cannot be elicited from any other animal. However, it would be reasonable to imagine that some species of experimental animal of those tried would react to excess iron with cirrhosis and pancreatic fibrosis. Furthermore, the dog would seem particularly likely to respond to excessive iron in a manner similar to that of man since many similarities in the iron metabolism of the two species have been defined.[42, 59, 70-74] However, species specificity cannot be ruled out by the data available.

Other possible causes for failure in the experimental induction of hemochromatosis might be related to inadequate dosage of iron and insufficient length of follow-up. Many investigators have employed amounts of iron below those found in patients displaying symptoms of idiopathic hemochromatosis, though our experiments and several others[14-17, 42] provided tissue iron concentrations comparable to those of the human disease. Furthermore, even higher doses of iron (2.5 to 3.5 Gm./Kg.) have failed to produce hemochromatosis in dogs which died in 6 to 9 months.[75] Premature sacrifice or inherently inadequate longevity may be cited as further objection to many experimental attempts. These are, indeed, valid objections in many cases, though the 4 years Polson followed his rabbits[12] and the 7-year period in the life of our dogs might be expected to telescope the aging changes to approximate the conditions produced by 45 years of iron overload in idiopathic hemochromatosis, the 20 to 40 years in the Bantu, and the 2- to 10-year period in patients receiving multiple transfusions.

Particular objection might be made to the schedule of iron administration used in the present study. It could be argued that giving the total amount of

iron within a few weeks, and then giving no more iron during the long period of observation, might somehow alter the tissue reaction to it. Giving small doses of iron throughout the entire period of observation might have changed its distribution and caused tissue damage. Several refutations to this type of objection are apparent. First, the same dose of a toxic substance is generally more likely to cause damage when given rapidly than when spread out over a long period of time. The rapidity of iron accumulation has been thought to accentuate the earlier onset of symptoms in young patients with idiopathic hemochromatosis[76] and in patients receiving multiple transfusions.[43] Second, if the supposition were made that prolonged administration of small doses of iron might eventually overwhelm the body's defenses or reparative processes, it would seem likely that short massive doses might accentuate this effect and be evident at the time of the first biopsy, but that was not the case. Third, investigators who used a technique of iron administration in which small increments were given for long periods produced results no different from ours.

The localization of the administered iron almost exclusively in the reticuloendothelial system of various experimental animals with little redistribution or spillover to the parenchymal cells might also be a factor in the failure of experimental production of this disease. Certainly, parenchymal distribution of iron is one of the hallmarks of idiopathic hemochromatosis. Iron introduced by the intravenous, intramuscular, or subcutaneous route is to a major degree first seized by R.E. cells which may effectively "lock it up" preventing its entrance into the parenchymal cells and eliminating critical tissue damage. Achieving appreciable parenchymal localization of iron in experimental animals has been a major difficulty, and has been seen best when iron has entered the body from gastrointestinal absorption. Unfortunately, the amounts of tissue iron have been smallest and the periods of follow-up generally the shortest in these experiments; hence, extension of this type of approach might be profitable. However, intraperitoneal injection should partially by-pass the reticuloendothelial barrier and experiments using this route have been unsuccessful in producing the lesions of hemochromatosis in mice,[69] rabbits,[12] and dogs.[75] Furthermore, iron from multiple transfusions in human beings apparently spills over from its predominantly reticuloendothelial storage sites in a small proportion of patients, independent of the number of transfusions or the duration of iron overload. In our dogs both the concentration of tissue iron and the duration of overload were greater than that of most of the reported human cases, and no similar spillover was seen. As will be pointed out later, factors in addition to iron in human beings may account for the difference.

Finally a healthy skepticism should be maintained regarding the role of iron as the sole cause of the changes of hemochromatosis. This skepticism has been voiced by many investigators[13-15, 25, 77, 78] though recently other authorities have disagreed.[43] Much of the reason for disagreement has stemmed from the nomenclature of diseases associated with excessive tissue iron deposits. However, in the idiopathic heredofamilial disease, increased absorption of iron and its deposition predominantly in the parenchymal cells of the liver and other organs of the body may be only a by-product of an inborn metabolic error.

The cirrhosis of the liver and the pancreatic and splenic fibrosis that accompany the iron deposition in these sites may not have been caused by the iron at all. The abundance and easy identification of tissue hemosiderin deposits may have blinded us to the subtle subcellular changes or the poorly studied biochemical alterations at the enzymatic level. Support for such an iconoclastic point of view is available from several observations. Foremost, of course, is the failure to induce the disease by iron administration to experimental animals despite all the qualifications just discussed. Of equal weight is the disparity between the amount of iron in tissues and reaction to it in the Bantus,[8-10] and in patients receiving multiple transfusions. Numerous studies of the Bantus have emphasized the differences between the clinical and pathologic features of their excessive iron deposition and those of patients with idiopathic hemochromatosis. There appears to be a complete lack of correlation between the amount of iron and cirrhosis, fatty changes in the liver, and pancreatic lesions in the Bantu. Likewise, in the few patients with transfusion hemosiderosis whose clinical course and tissues at autopsy are indistinguishable from those of the idiopathic disease, factors other than excessive tissue iron may play a major role. Some of these factors may be chronic anemia, frequent febrile transfusion reactions, viral hepatitis, and severe nutritional disturbance. Still other entirely unknown factors that allow the transfer of iron from reticuloendothelial deposits to parenchymal cells may be necessary.

The suggestion that iron alone may not be the cause for the tissue damage in these various conditions does not eliminate the possibility that excessive tissue iron may aggravate damage initiated by other factors as yet unknown. Indeed, it would be difficult to imagine any material, however inert, which would not exert harmful effects if present in tissues in large enough concentrations.

Thus, until the fundamental cellular derangements of hemochromatosis can be delineated and the pathogenesis of the fibrosis and the specific localization of iron as well as the cause for its excessive absorption defined, it might be more profitable to shift attention from the large tissue iron accumulations to the enzyme systems of the involved parenchymal cells in an effort to determine why iron localizes there and what effect it exerts.

SUMMARY

The effects of iron overload in dogs were studied for prolonged periods (4 to 7 years). Four animals were given iron intravenously as saccharated iron oxide; two were transfused with whole blood. The total dose was 0.5 or 1.0 Gm. of iron per kilogram of body weight.

No evidence of cirrhosis could be found by liver function tests or by histologic sections at biopsy or autopsy. There was no diabetes or pancreatic fibrosis. Heavy iron deposits were found predominantly in reticuloendothelial cells. There was no evidence of any fibrous tissue reaction.

Blindness developed in all of the animals, the eye lesions resembling those of retinitis pigmentosa. With this exception the tissue iron overload was remarkably well tolerated.

Hematologic changes were plethora (in the transfused dogs) and serum iron elevations to values 20 times normal.

From the chemical estimation of the tissue iron in biopsy and autopsy specimens there is no evidence that redistribution of the iron load occurred. From 37 to 73 per cent of the administered iron could be accounted for.

The failure to produce hemochromatosis with the large amounts of iron injected and the prolonged period of observation leads to the following conclusions:

1. The manner of administering the iron to these animals allowed the reticuloendothelial cells to remove it effectively and prevent parenchymal localization.

2. There may be a species difference between man and the dog which makes it possible for the dog to tolerate easily amounts of iron which in man might produce hemochromatosis.

3. The role of iron in causing the tissue damage of hemochromatosis may well have been overemphasized, e.g., iron may simply be deposited at the site of tissue injury which has been initiated by other agents.

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STUDIES OF ERYTHROCYTE OSMOTIC FRAGILITY IN IRON DEFICIENCY ANEMIAS

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MORPHOLOGICALLY, there is no certain way to distinguish iron deficiency anemias from the trait form of hereditary leptocytosis (thalassemia minor), since in both conditions examination of stained smears of the peripheral blood will reveal hypochromasia, microcytosis, and often, "target" erythrocytes. Basophilic stippling may be present in either condition. It is well known that the erythrocytes of patients who have hereditary leptocytosis show an increased resistance to hemolysis in decreasing strengths of hypotonic solutions of sodium chloride,[1] and the demonstration of this fact has been cited as evidence supporting the diagnosis of this condition.

This study is an attempt to assess whether or not studies of erythrocyte osmotic fragility, done routinely, are of help in distinguishing the anemia of iron deficiency from the trait form of hereditary leptocytosis.

METHODS AND MATERIALS

Twenty-seven patients with hypochromic microcytic anemias of known causation were selected for study. In each instance a cause for iron deficiency seemed apparent; the vast majority of patients had an obvious source of chronic loss of blood. Specimens of the blood of each of the 27 patients were then subjected to a study of erythrocyte osmotic fragility, and in 13 cases reticulocyte counts also were performed.

By the method used in the laboratories of the Mayo Clinic the normal range of hemolysis of erythrocytes in hypotonic solution of sodium chloride extends from 0.44 per cent solution of sodium chloride (initial hemolysis) to 0.30 per cent solution of sodium chloride (complete hemolysis).

RESULTS

The results of studies of erythrocyte osmotic fragility can be seen in Table I. In all but one of the 27 patients, hemolysis continued beyond 0.30 per cent solution of sodium chloride and in 10 patients hemolysis was incomplete at 0.28 per cent, at which concentration the test was discontinued. It should also be noted that in 10 cases, hemolysis did not appear until a dilution of greater than 0.44 per cent solution of sodium chloride was used, and in 2 cases hemolysis did not occur until a dilution of 0.36 per cent solution of sodium chloride had been reached. It is of interest that in 5 cases there seemed to be some increase in fragility, for initial hemolysis appeared in dilutions of less than 0.44 per cent solution of sodium chloride.

COMMENT

This study clearly demonstrates that the erythrocytes of patients with iron deficiency anemia exhibit an increased resistance to hemolysis in decreasing

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strengths of hypotonic solution of sodium chloride. Reimann and Arkun,[2, 3] in a similar study of 21 cases of iron deficiency anemia, also were able to demonstrate such an increased resistance. In addition, they observed that the curve of

TABLE I. SOURCE OF BLOOD LOSS, MORPHOLOGIC ASPECTS OF PERIPHERAL BLOOD AND RESULTS OF STUDIES OF ERYTHROCYTE OSMOTIC FRAGILITY IN 27 CASES OF IRON DEFICIENCY BLOOD LOSS

CASE	DIAGNOSIS	MORPHOLOGY IN STAINED SMEAR OF PERIPHERAL BLOOD	RETICULOCYTE COUNT (PER CENT)	FRAGILITY TO HYPOTONIC SOLUTION OF SODIUM CHLORIDE (RANGE IN PER CENT)
1.	Indeterminate lesion, left kidney	Marked hypochromic, microcytic picture		0.42 to 0.28 (incomplete)
2.	Multiple peptic ulcers	Severe hypochromasia, considerable polychromasia		0.42 to 0.28 (incomplete)
3.	Carcinoma, cecum	Marked hypochromasia, microcytosis		0.44 to 0.28
4.	Multiple peptic ulcers	Moderate hypochromasia		0.46 to 0.28
5.	Prematurity, with hypochromic anemia	Severe hypochromic, microcytic picture		0.50 to 0.28 (incomplete)
6.	Postgastrectomy iron deficiency anemia	Hypochromic microcytic picture		0.46 to 0.28
7.	Peptic esophagitis, with ulceration	Severe hypochromasia		0.46 to 0.28
8.	Hypernephroma	Moderate hypochromasia		0.44 to 0.28
9.	Hemorrhagic gastrojejunal ulcer	Severe hypochromic, microcytic picture		0.36 to 0.28 (incomplete)
10.	Nontropical sprue	Hypochromasia and polychromasia		0.44 to 0.28 (incomplete)
11.	Chronic duodenal ulcer, bleeding	Microcytic, hypochromic picture		0.38 to 0.28
12.	Postgastrectomy iron deficiency anemia	Marked hypochromic, microcytic picture		0.44 to 0.28 (incomplete) (serum iron 5 mg.)
13.	Gastric ulcer	Hypochromasia, microcytosis		0.46 to 0.30
14.	Chronic ulcerative colitis	Moderate hypochromasia	1.7	0.42 to 0.28
15.	Chronic ulcerative colitis	Moderate hypochromasia	6.7	0.40 to 0.28 (incomplete)
16.	Chronic ulcerative colitis	Hypochromasia, polychromasia	4.6	0.42 to 0.28
17.	Bleeding diaphragmatic hernia	Moderate hypochromasia, polychromasia		0.42 to 0.28
18.	Bleeding duodenal ulcer	Moderate hypochromasia	2.9	0.44 to 0.28 (incomplete)
19.	Carcinoma, right colon	Hypochromasia with polychromasia	2.4	0.42 to 0.28
20.	Iron deficiency anemia due to menorrhagia	Moderate hypochromasia	2.9	0.36 to 0.28
21.	Menorrhagia, anemia	Moderate hypochromasia, slight microcytosis	1.9	0.44 to 0.28 (incomplete)
22.	Diverticulitis	Marked hypochromasia	0.5	0.44 to 0.28
23.	Abdominal carcinomatosis	Mild hypochromasia	1.6	0.44 to 0.28
24.	Hereditary hemorrhagic telangiectasia, loss of blood	Moderately severe hypochromasia, polychromasia	11	0.44 to 0.28
25.	Chronic ulcerative colitis	Moderate hypochromasia	2.9	0.40 to 0.28 (incomplete)
26.	Chronic ulcerative colitis	Moderate hypochromasia	5.2	0.44 to 0.28
27.	Incomplete abortion, hemorrhage	Hypochromic, microcytic smear	2.5	0.40 to 0.28

hemolysis in this condition often is spread beyond the normal on both sides, so that increased fragility also may be present. This occurred in some cases studied in this series, although the degree was not striking.

The physiologic basis for increased resistance to hypotonic solutions of sodium chloride is not perfectly understood. Dameshek and Schwartz[4] and Stephens[5] pointed out in separate papers that the reticulocyte is less fragile than the more mature erythrocyte, and, therefore, is more resistant to hemolysis in decreasing strengths of hypotonic solutions of sodium chloride. Other workers, however, feel that the opposite more probably is true.[6, 7] In many of the cases in the present series reticulocytosis was present, but it is doubtful that this could explain the increased resistance to hypotonic solutions of sodium chloride which was present.

Numerous investigators[8-10] have found that the erythrocyte serves as a perfect osmometer. Since the erythrocyte in iron deficiency anemias possesses a surface area proportionately greater than its cellular contents, the ratio of volume-to-surface area is low, and increased osmotic resistance results.

Whatever the cause, suffice it to say that increased osmotic resistance to hypotonic solutions of sodium chloride definitely can be demonstrated in the presence of iron deficiency anemia. Therefore, the demonstration of increased resistance by routine studies of erythrocyte osmotic fragility is of little or no value in attempts to distinguish the trait form of hereditary leptocytosis from iron deficiency anemias.

SUMMARY

Studies of erythrocyte osmotic fragility were performed in 27 cases of iron deficiency anemia of known causation. It was adequately demonstrated that increased osmotic resistance to hypotonic solutions of sodium chloride occurs in this type of anemia. Studies of erythrocyte osmotic fragility, therefore, are of little or no value in attempts to distinguish the trait form of hereditary leptocytosis from iron deficiency anemias.

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URINARY EXCRETION OF ACID MUCOPOLYSACCHARIDES BY PATIENTS WITH LUPUS ERYTHEMATOSUS

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SEVERAL diseases, including lupus erythematosus and rheumatic fever, have inflammatory lesions of connective tissue. It has been shown that acid mucopolysaccharides (APS) are present not only as important constituents of connective tissue[1] but occur in the blood[2] and urine.[3, 4] The APS of normal human urine consists almost entirely of chondroitin sulfate and hyaluronic acid.[5, 6] The amount excreted per day has been determined for normal adults and children,[3, 4, 7] and increased excretion of APS has been observed in patients with rheumatoid arthritis.[6, 8] This study shows that the APS excretion by patients with lupus erythematosus is much greater than normal. It is suggested that the observed changes in the rate of APS excretion may be related to the inflammatory processes characteristic of this disease.

MATERIALS AND METHODS

This study was conducted on 5 women between the ages of 19 and 50, who had had the typical signs and symptoms of lupus erythematosus for periods varying between 5 months and 10 years. The L. E. cell phenomenon was sharply and repeatedly positive except in one patient (A. Y.) where it was weakly positive on two occasions. In each case the patients were hospitalized because of high fever and acute polyarthritis. Three of the patients (F. B., R. deA., and N. G.) also had joint changes of the type seen in rheumatoid arthritis but in no patient was there either an abnormal increase in sensitized sheep cell agglutinins or a positive gamma globulin precipitation test.[9]

Three of the 5 patients had renal involvement. In two (F. B. and N. G.) it was mild and was manifested only in slight albuminuria, microscopic hematuria, and cylindruria. One patient (R. deA.) had the nephrotic syndrome with severe albuminuria and elevated serum lipids. None of the patients had an elevated level of blood urea or decreased urea clearance.

All of the patients had abnormal patterns of serum protein when first seen, with low albumin and elevated gamma globulin. These became more normal during therapy although the level of albumin remained below normal in the case of R. deA., probably because of loss through the kidneys.

The treatment received during the study is indicated in Table I and Fig. 1.

Urinary acid mucopolysaccharides were measured by the method of Di Ferrante and Rich[4] and the results expressed as milligrams of glucuronic acid per 24 hours. The erythrocyte sedimentation rate was measured by the Westergren method.

RESULTS

In all patients studied the urinary excretion of APS was greater than the normal for adult women of 2.7 to 5.4 mg. per 24 hours (Table I). The detailed

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clinical course as indicated by signs and laboratory determinations is compared with the APS excretion for three of the patients in Fig. 1. F. B. showed a progressive decline in the urinary APS excretion to near normal levels during treatment with prednisone, and during a time when she had a clinical remission

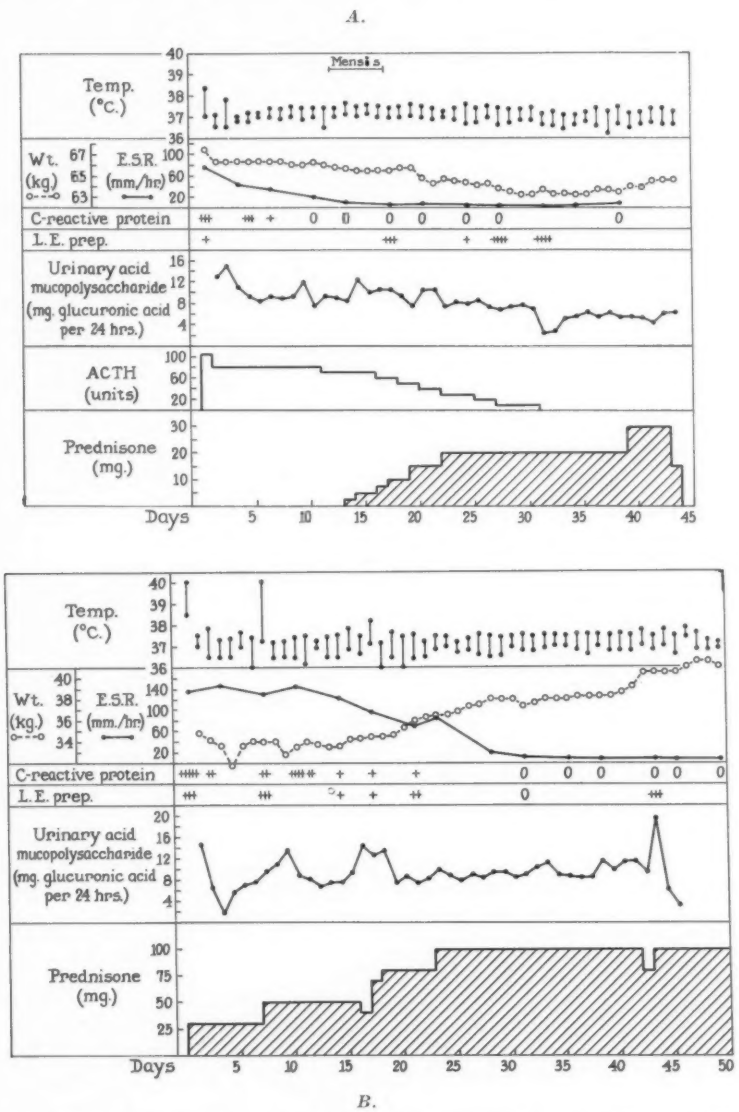

Fig. 1.—The clinical course and rate of acid mucopolysaccharide excretion by three women with systemic lupus erythematosus. *A*, F. B. (50 years old). *B*, M. M. (32 years old). *C*, R. deA. (20 years old).

(Fig. 1*A*). The changes in rate of APS excretion were gradual, and occurred later than those in the other laboratory tests indicated. In the other patients there was no similar sustained decrease in the rate of APS excretion either during treatment or improvement in clinical status. In M. M. (Fig. 1*B*), there was a sharp temporary rise in APS excretion on two occasions at the time

of an exacerbation of the disease as shown by increased symptoms and elevation of temperature. In both M. M. and R. deA. there was temporary elevation in rate of APS excretion associated with reduction in the amount of medication (Figs. 1*B* and 1*C*). Except as indicated, there was no change in the urinary APS excretion related to the clinical course or to the type of treatment.

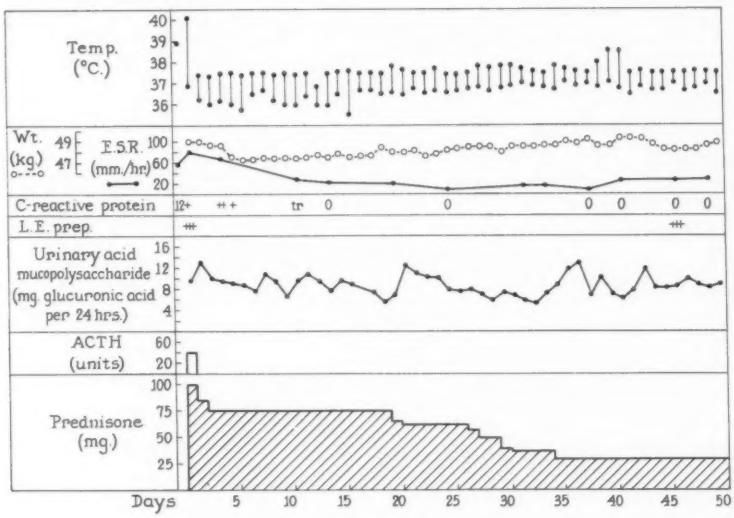

Fig. 1*C*. *(For legend see opposite page.)*

TABLE I. SUMMARY OF RATES OF URINARY ACID MUCOPOLYSACCHARIDE EXCRETION IN 5 PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

PATIENT	SEX	AGE	INITIAL URINARY ACID MUCOPOLYSACCHARIDE*	MEDICATION RECEIVED DURING PERIOD OF STUDY	VARIATION IN URINARY ACID MUCOPOLYSACCHARIDE DURING TREATMENT
F. B.	F	50	Elevated (8 to 16)	See Fig. 1*A*.	Declined gradually to near normal levels.
M. M.	F	32	Elevated (5 to 15)	See Fig. 1*B*.	Declined to range of 6 to 10 with peaks of 14 to 20 which occurred during exacerbations of the disease
A. Y.	F	27	Slightly elevated (6 to 8)	Prednisone, 30 mg. per day rapidly increased to 75 mg. per day and maintained.	No change. Remained slightly above normal, between 6 and 10
R. deA.	F	19	Elevated (9 to 14)	See Fig. 1*C*.	Gradual downward trend during treatment but with temporary increase after reduction in the dose of prednisone
N. G.	F	39	Slightly elevated (6 to 7)	Aspirin, 0.6 Gm. per day increased to 3.6 Gm. per day. Chloroquine diphosphate, 500 mg. per day.	Rose to 9 to 13 during treatment with aspirin. Returned to range of 6 to 8 during combined aspirin and chloroquine therapy

*Expressed as milligrams glucuronic acid per 24 hours. Normal range for adult women is 2.7 to 5.4.

DISCUSSION

Because of the severity of the illness it was not possible to study these patients before treatment was started. In the 5 patients studied, there was a moderate to very marked elevation of the APS excretion which appeared to be little affected by the clinical course or type of treatment. This is at variance with the finding that the elevated urinary APS excretion by patients with rheumatoid arthritis returns to normal during treatment with salicylate[6] or cortisone.[10] The different response of urinary APS excretion to treatment in these two diseases may be due to the presence of renal involvement in lupus erythematosus, but more probably reflects a fundamental difference in the metabolic abnormality of connective tissue in these two states.

SUMMARY

1. In 5 patients with lupus erythematosus, the daily rate of urinary acid mucopolysaccharide (APS) excretion has been measured for more than one month during which several forms of treatment were used and a clinical remission obtained.

2. In each patient the rate of APS excretion was above normal. Minor variations in rate could be related in a few instances to changes in the clinical state of the patient or to the amount or type of treatment. However, no consistent response to therapy was observed, and in no instance did the APS excretion return to normal during the period of study.

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LABORATORY METHODS

THE DISTRIBUTION OF TOXINS IN COAGULASE-POSITIVE STAPHYLOCOCCI ISOLATED FROM INFECTIONS AND CARRIERS

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THE pathogenic staphylococci are well known for their ability to elaborate a wide variety of antigenic substances, many of which have been designated exotoxins. The use of this latter term implies a direct participation of the diffusible antigen in question in that interplay between host and parasite which results in typical staphylococcal infection, a relationship that is frequently not readily apparent.

This study was undertaken in an attempt to determine the distribution of alpha, beta, and delta hemolysins and of staphylokinase in coagulase-positive staphylococci and the relationship of these various components to the virulence of these organisms.

In nature, the Staphylococcus exists as a parasite on the mucosa of the nasopharynx and the skin of man and characteristically produces more or less destructive localized lesions, particularly of skin and subcutaneous tissues. Two classes of overt infection are commonly encountered; those arising in grossly normal tissue or following relatively minor insult as illustrated by the furuncle or carbuncle, and those following the invasion of significant traumatic or operative wounds.

It does not seem unreasonable to assume that if the main reservoir of staphylococci in nature is in the nasopharynx of man, the collective characteristics of a group of strains isolated from this site would encompass the entire range and distributional frequency of occurrence of those factors related to virulence.

Further, strains of staphylococci which, when deposited under those ill-defined conditions which allow primary implantation in grossly normal tissue, can survive, multiply, and produce a necrotizing lesion—regardless of the ultimate severity of that lesion—must possess to an important extent those factors related to virulence.

Organisms isolated from infected wounds, ulcers, tumors, and burns constitute a further group. There is no doubt that fully virulent pathogens may be responsible for such infections. It is just as evident, however, that in the presence of devitalized tissue in a debilitated host, organisms of lesser virulence

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are quite capable of implantation and the production of significant infection. This group, therefore, may well represent a more heterogeneous collection than that composed of organisms isolated from furuncles and carbuncles and may be more likely to contain a proportion of strains of considerably lesser virulence.

We propose to base our classification of strains with respect to virulence on this rationale.

MATERIALS AND METHODS

Strains of Staphylococci.—Three hundred and sixty strains of coagulase-positive staphylococci were isolated from cases in the Kingston General Hospital and 210 strains from healthy nasal carriers. In all instances, single colony picks were made, coagulase tests carried out by the slide method, and the strains stored on nutrient agar slopes at 5° C. with subcultures being made at monthly intervals. All strains were tested within 3 to 4 weeks of primary isolation and all were subsequently checked at 3 to 6 months. No remarkable changes in toxin production were noted in the storage period.

Hemolysin Assay.—The production of alpha, beta, and delta toxins was determined by the antitoxin strip technique of Elek and Levy,[1] using antitoxin prepared by the Connaught Laboratories (Toronto). The strains were divided arbitrarily into strong, moderate, and weak toxin producers on the basis of the size of the zone of hemolysis.

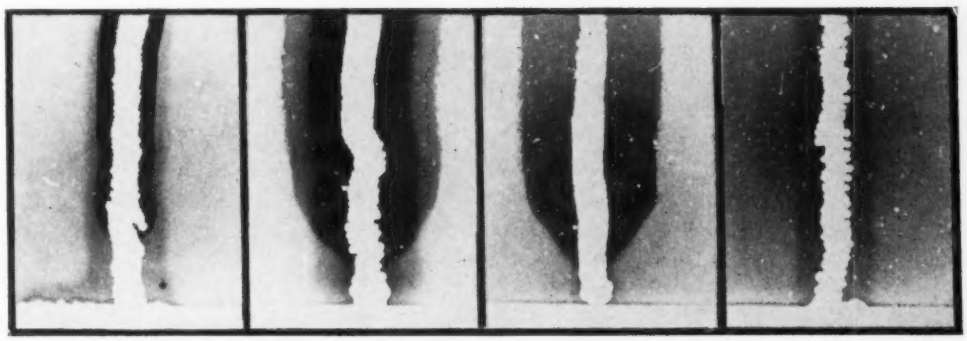
Staphylokinase Assay.—Staphylokinase production was tested by a modification of the plasma plate method of Christie and Wilson.[2] Sterile human oxalated plasma was added in a concentration of 15 per cent to melted nutrient agar and heated at 55° C. for 15 minutes in order to precipitate the fibrinogen. This turbid agar was then poured into plates. If such agar is spot-inoculated with staphylococci, zones of clearing appear around some of the strains and this has been taken as evidence of the production of staphylokinase.

Lack[3] has pointed out that the clearing of this agar can be due to at least two factors, the activity of staphylococcal protease or the activation of human plasminogen by staphylokinase. Soybean trypsin inhibitor (S.B.I.) will inhibit staphylokinase-activated plasmin but does not affect the activity of bacterial protease. Lack thus suggested that two plasma plates be used for this test, one with S.B.I. added and one without. True staphylokinase-producing strains induce clearing only in the absence of S.B.I.

During the course of our investigation a double zone of clearing was noted in the case of a number of strains, an outer partially clear zone and an inner translucent zone. This double zone was seen only in the absence of S.B.I. When S.B.I. was present only a single zone of partial clearing was observed. This suggested that a number of strains produced both staphylokinase and staphylococcal protease. A procedure was devised in order to detect more easily the presence of both of these components. Heated plasma plates were prepared as above. S.B.I. in a concentration of 2 mg. per milliliter in phosphate buffer at pH 7.7 was stored at −20° C. A filter paper strip 75 mm. by 10 mm. cut from Reeve Angel paper No. 202 was soaked in the S.B.I. solution and laid across the plate which was then dried for one hour in the incubator. The various strains were inoculated as streaks at right angles to the filter strip and the plates incubated at 37° C. for 48 hours. Typical results are indicated in Fig. 1. Staphylococcal protease produces a relatively narrow zone of partial clearing which extends right up to the strip. Staphylokinase produces a zone of partial clearing which is distinctly inhibited by the S.B.I. diffusing from the filter strip. When both are present translucent clearing of the agar occurs within the area of double activity. An interesting example is shown in Fig. 1, *A* of a zone of staphylokinase activity occurring completely within the zone of protease activity and indicated only by the inhibition of the translucent area of double activity.

Total Diffusible Antigens.—Together with the identification of the three hemolysins and staphylokinase, it was considered to be of value to estimate the number of diffusible antigens produced by the strains under study.[4] The heart infusion basal medium described by Elek[1] was poured into Petri dishes in 12 ml. amounts and allowed to harden. A filter-paper strip

soaked in staphylococcus antitoxin was placed across each plate and the plate dried in the incubator for one hour. The strains were inoculated at right angles to the strip and incubated for 48 hours in a 30 per cent CO_2–70 per cent air mixture. A final reading of the plates was made after a further 48 hours at 5° C. Distinct antigen-antibody flocculation lines which appeared were counted using a hand lens and from the number of lines an estimate was made of the minimum number of diffusible antigens produced by each strain. Three of the line patterns are illustrated in Fig. 2. The number of lines produced is, of course, basically limited by the number of distinct antibodies present in the commercial antiserum.



A. *B.* *C.* *D.*

Fig. 1.—The qualitative determination of staphylokinase production. *A* and *B*, Staphylokinase and protease activity. *C*, Staphylokinase alone. *D*, Protease alone.

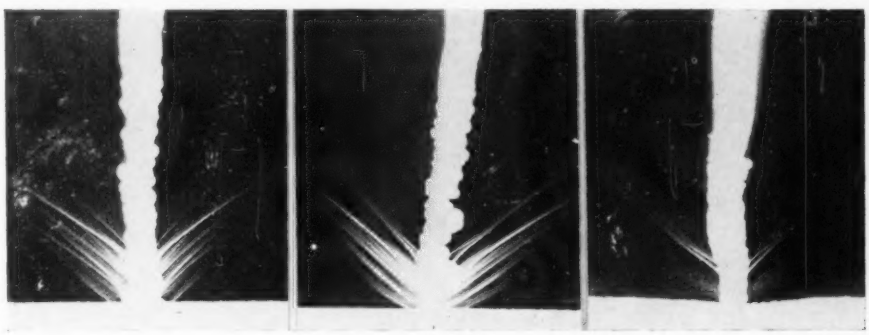


Fig. 2.—Determination of diffusible antigen production. Three representative patterns of antigen-antibody flocculation lines.

Grouping of the Strains Under Study.—The strains isolated were divided into 5 groups which we felt best reflected an average gradation of virulence, according to the concepts previously outlined.

Group I: Organisms isolated from primary, closed, staphylococcal lesions; namely, boils and carbuncles, a collection of virulent pathogens.

Group II: Organisms isolated from infected wounds, burns, ulcers, and tumors, a more heterogeneous collection than Group I and more likely to contain a proportion of strains of low virulence.

Group III: Organisms isolated from nasal and pharyngeal swabs, sputum and urine. A great many of these strains were considered to have no etiologic relationship to any disease in the patient and most were felt to represent *patient carrier* strains. This group would be likely to be even more heterogeneous with respect to virulence than Group II.

TABLE I. NUMBER OF ANTIGEN-ANTIBODY FLOCCULATION LINES PRODUCED BY COAGULASE-POSITIVE STAPHYLOCOCCI ISOLATED FROM DIFFERENT GROUPS

GROUP	NO. OF STRAINS TESTED	PER CENT OF STRAINS PRODUCING THE INDICATED NUMBER OF LINES													
		0	1	2	3	4	5	6	7	8	9	10	11	12	13
I. Primary closed infections	121	0	0	0	0	0.9	3.4	17.1	18.8	14.5	26.5	12.8	4.3	1.7	0
II. Wound infections	110	0	0	0	0	0.9	5.6	17.8	20.6	24.6	12.8	11.2	4.7	1.9	0
III. Patient carriers	151	0.7	1.4	1.4	2.8	4.2	16.0	20.1	16.0	18.0	9.7	4.2	4.2	1.4	0
IV. Hospital staff carriers	122	0	0	0.8	0.8	2.5	5.7	18.0	17.2	23.8	17.2	8.2	3.3	1.6	0.8
V. University student carriers	105	1.9	1.9	2.9	2.9	4.8	19.0	16.2	21.9	14.3	5.7	6.7	1.9	1.0	

Group IV: Organisms isolated from the nasopharynges of hospital staff members. These represent a group of carrier strains obtained from personnel exposed to a rather special and selective environment.

Group V: Organisms isolated from the nasopharynges of university students having no remarkable hospital contact. These strains were considered representative of the over-all distribution of strains with respect to virulence, as they are found in their primary reservoir in nature.

RESULTS

Analyses of the significance of the differences in distribution of the factors considered in the following tables were carried out by a X^2 test and only when $p < 0.5$ is the term significant used.

Diffusible Antigen Production.—An over-all survey of the ability of the strains under study to elaborate diffusible antigens is presented in Table I. Group III is seen to consist of a rather heterogeneous group with a distribution of the number of antigens produced from 0 to 12. The distribution of Group III strains is not significantly different from that of Group V strains which are taken to represent a cross section of the staphylococci in their natural habitat, the nasopharynx of man.

Group II strains constitute a more homogeneous group with a significant proportion of the strains producing more diffusible antigens than those in Group III or the strains derived from normal carriers.

Group I strains are also shown to be rather homogeneous and as a group demonstrate the greatest faculty for diffusible antigen production.

The strains isolated from carriers constantly exposed to the hospital environment show a distribution in respect to antigen production which is not significantly different from Group II. These strains, however, produce on the average considerably more antigens than Groups III and V and significantly less than I.

Thus, the groups in question may be arranged in order of their ability to elaborate diffusible antigens from greatest to least as follows, I, II and IV, III and V.

It should be noted that because two strains produce the same number of antigens under the conditions of this test, this does not mean that the antigens produced are necessarily the same. Nor have we any way of knowing at the present time what antigen any particular line represents. It seems likely, however, that the conventional staphylococcal exotoxins are represented among these diffusible antigens and that a strain producing a large number of lines is likely to be a significantly toxigenic strain.

Production of alpha, beta, and delta Hemolysins and Staphylokinase.—The ability of the various strains to produce four specific diffusible components, alpha, beta, and delta hemolysin and staphylokinase, is indicated in Table II. Table III illustrates to some extent the degree of in vitro toxigenicity.

Groups I, II, and III are arranged in order of descending toxigenicity with respect to the production of alpha and delta hemolysins and staphylokinase. This is most evident with alpha toxin and staphylokinase. Group I strains are distinctly more delta toxigenic than II and III although the differences

between these two latter groups is not significant. Group V strains, obtained from normal carriers, would appear to be the least toxigenic and related closely to Group III. Group IV strains appear to be located toxigenically between I and II.

TABLE II. THE INCIDENCE OF ALPHA, BETA, AND DELTA HEMOLYSINS AND STAPHYLOKINASE IN 570 STRAINS OF COAGULASE-POSITIVE STAPHYLOCOCCI ISOLATED FROM DIFFERENT GROUPS

GROUP	NO. OF STRAINS TESTED	PER CENT POSITIVE			
		α-TOXIN	δ-TOXIN	β-TOXIN	STAPHYLOKINASE
I. Primary closed infections	119	100	99.1	30.3	97.4
II. Wound infections	110	96.4	88.1	29.9	86.3
III. Patient carriers	131	86.2	88.5	30.4	84.0
IV. Hospital staff carriers	100	96.0	92.0	51.0	90.0
V. University student carriers	110	89.1	78.2	19.9	71.1

TABLE III. THE DISTRIBUTION OF STRONG, MODERATE, WEAK, AND NONTOXIN PRODUCERS AMONG COAGULASE-POSITIVE STAPHYLOCOCCI ISOLATED FROM DIFFERENT GROUPS

GROUP	ALPHA TOXIN				DELTA TOXIN			BETA TOXIN			
	+++	++	+	0	++	+	0	+++	++	+	0
I. Primary closed infections	86.9	9.0	4.1	0	67.7	31.4	0.9	5.0	9.6	15.7	69.7
II. Wound infections	70.0	14.5	11.9	3.6	62.6	25.5	11.9	5.5	13.6	10.9	70.1
III. Patient carriers	51.0	21.1	14.1	13.8	60.0	28.5	11.5	5.3	10.0	15.1	69.6
IV. Hospital staff carriers	82.0	6.0	8.0	4.0	72.0	20.0	8.0	12.0	24.0	15.0	49.0
V. University student carriers	41.8	30.9	16.4	10.9	50.0	28.2	21.8	6.4	6.4	7.4	80.0

TABLE IV. THE DISTRIBUTION OF COMBINATIONS OF TOXINS PRODUCED BY COAGULASE-POSITIVE STAPHYLOCOCCI

GROUP	TOXIN COMBINATION IN PER CENT												NUMBER TESTED
	ADF	ABDF	AF	AD	ABD	A	ABF	DBF	DF	D	DB	0	
I. Primary closed infections	67.2	29.4	0.9	1.7	0.9	0	0	0	0	0	0	0	119
II. Wound infections	55.5	21.8	4.5	5.6	3.5	2.8	2.8	1.8	0	0	0	1.8	110
III. Patient carriers	52.7	24.3	3.1	1.5	3.8	0.8	0	0.8	3.1	0.8	1.5	7.6	131
IV. Hospital staff carriers	35.0	49.0	5.0	6.0	1.0	0.0	0	1.0	0	0	0	3.0	100
V. University student carriers	41.8	13.6	8.2	10.9	4.5	8.2	1.8	0	5.6	1.8	0	3.5	110

A = alpha toxin; D = delta toxin; B = beta toxin; F = staphylokinase.

Table IV indicates the distribution of combinations of toxins produced by the organisms isolated from the several groups. The predominant strains found in all the groups are those which produce alpha and delta lysin and staphylokinase with or without beta lysin. Group I is seen to be most homogeneous in this respect with no less than 96.6 per cent of the strains producing alpha and delta lysin and staphylokinase. Group II is somewhat more heterogeneous and Group III is the most heterogeneous of all with 11 different combinations being represented. Group V resembles Group III while Group IV strains derived from the nurses fall somewhere between Groups I and II. It is interesting to note the relative rarity of pure alpha or delta producing strains and the absence of strains which produce beta lysin or staphylokinase in the absence of the other 3 components.

The results with respect to beta toxin as seen in Tables II and III do not follow the pattern of distribution noted for the other three products. No difference is noted in the incidence of beta toxin producers in any of the three groups of strains isolated from hospitalized patients. However, a lower incidence is noted in the group isolated from the normal carriers and a strikingly greater incidence, no less than 50 per cent in the group isolated from hospital staff carriers.

One may speculate that a strain of Staphylococcus with a particular biologic character may become established in the hospital as a predominant strain. The nurses, living in this environment, would be more likely to carry a strain having this character, although the character in question may be completely unrelated to virulence. Such may be the explanation of the distribution of beta toxigenic strains in the groups studied. The greater number of beta positive strains in the three groups isolated from hospital patients, as compared to the normal carriers, may be evidence merely of infection of those patients with the hospital strain and not evidence in favor of an important role for beta toxin in the pathogenesis of staphylococcal disease.

TABLE V. A COMPARISON OF THE BETA TOXIN PRODUCTION BY STAPHYLOCOCCI ISOLATED FROM DIFFERENT GROUPS

GROUP	PER CENT BETA TOXIN POSITIVE	
	HOSPITAL INFECTIONS	NONHOSPITAL INFECTIONS
I. Primary closed infections	44.1	24.1
II. Wound infections	56.0	22.4
IV. Hospital staff carriers	51.0	–
V. University student carriers	–	19.9

Strong support for the hypothesis outlined above can be seen in Table V. Strains derived from infections arising in hospital and from the nurses' nasal cultures show a remarkable incidence of beta toxin production, whereas those strains derived from normal carriers and nonhospital infection show a considerably lower incidence. Group III has been excluded as there is no way of knowing whether a strain isolated from a patient's throat was present on admission or was implanted subsequently. No remarkable relationship between beta toxin production and the standard grouping is noted.

The demonstration that a particular toxigenic variety of staphylococcus predominates in our particular hospital introduces a possible objection to our major thesis. It may be argued that the diminishing toxigenicity seen in Groups I, II, and III, with respect to alpha and delta toxin and staphylokinase, may be merely an indication of the proportion of hospital infections in each group. The strains predominant in the hospital may, by adventitious selection, be more toxigenic and thus the more hospital infections in a given group the more toxigenic that group would appear.

The answer to this objection would necessitate a study of the distribution of toxins in strains isolated from normal carriers and from infections arising in patients in their homes, and Table VI has been prepared from this point of view. It consists of a modification of Table II in which only those strains isolated from outpatients or from hospitalized patients immediately after admission have been retained. Group III must of necessity be excluded because the ultimate origin of the strains cannot be determined in any way. The nurses' strains (Group IV) are, of course, excluded.

TABLE VI. A COMPARISON OF THE TOXIN PRODUCTION OF STAPHYLOCOCCI ISOLATED FROM NORMAL CARRIERS AND FROM DISEASE ARISING OUTSIDE OF HOSPITAL

GROUP	NO. OF STRAINS	PER CENT POSITIVE			
		α-TOXIN	δ-TOXIN	β-TOXIN	STAPHYLOKINASE
I. Primary closed infections	83	100	100	24.1	98.8
II. Wound infections	85	96.5	89.4	22.4	87.1
V. University student carriers	110	89.1	78.2	19.9	71.1

An inspection of Table VI reveals that not only is the previously observed relationship between group and toxigenicity preserved, but also that this relationship becomes even more obvious.

DISCUSSION

The group of strains isolated from furuncles and carbuncles has been shown to produce significantly more diffusible antigens, alpha and delta lysin and staphylokinase than the group isolated from wound infections. This, in turn, has been shown to be more toxigenic than the group isolated from carriers.

The thesis has been advanced above that the division of the strains used in this work represents a division into groups in order of a diminishing average virulence. Elek[5] has recently demonstrated the striking importance of host factors in the pathogenesis of staphylococcal infections. The ability of a given strain to produce experimental skin lesions in human subjects was found to be enhanced more than five-hundred fold when a foreign body reaction was induced at the site of inoculation. This observation would appear to support our contention that strains isolated from furuncles would be more likely to be fully virulent than strains isolated from wound infections where gross disturbances in tissue defense mechanisms play an important role in the pathogenesis of the infection. If it is accepted that these groups represent a gradation in virulence, then it is apparent that a direct association exists between virulence

and multiple antigen production, alpha and delta lysin production, and staphylokinase elaboration. Elek and Levy[6] have suggested that the coagulase-positive staphylococci characteristically produce the full complement of hemolysins and that any other pattern represents a loss of one or more characters. The homogeneous nature of Group I strains would tend to support this contention and to imply that the production of alpha and delta lysin and staphylokinase is characteristic of fully virulent coagulase-positive staphylococci and any other pattern represents a degradation in virulence.

Some support for this view may be found in the literature. Schwabacher and associates[7] demonstrated that nonalpha toxin-producing strains were more common among those strains isolated from carriers than among those derived from lesions. Lesion-producing staphylococci have been shown to produce alpha toxin consistently[8] and to elaborate more alpha and delta lysin[9] and more antigen-antibody flocculation lines[10] than strains isolated from carriers. Howard[4] has demonstrated that the relative virulence of staphylococci for mice is associated with the number of diffusible antigens produced and the degree of completeness of the hemolysin pattern.

A number of workers have noted, however, the occurrence of nontoxigenic strains of coagulase-positive staphylococci. Lack[11] has recorded that no less than 9 of a series of 49 strains isolated from cases of osteomyelitis failed to produce alpha toxin. Further, a large proportion of patients with staphylococcal osteomyelitis failed to produce significant amounts of antihemolysin. It is significant to note that Lack observes that about 25 per cent of his strains were derived "from postoperative infections where foreign bodies such as sutures, steel pins, and foreign grafts have probably had the effect of greatly reducing the efficiency of host defenses. In such circumstances toxin production may not be necessary for the initiation of infection." There can be no doubt that host factors play a remarkable part in the pathogenesis of staphylococcal infection. None the less, it seems reasonable to profess that the ability to produce large amounts of toxin may determine whether a strain can initiate infection under conditions in which host resistance is not remarkably depressed. We are not suggesting that toxigenicity is the sole determinant of the pathogenicity of staphylococci, but only that the production of alpha and delta toxins and staphylokinase is characteristic of the more virulent strains.

The results with respect to beta toxin are of considerable interest. Many studies have shown that particular biotypes of coagulase-positive staphylococci tend to be selected and to predominate in the hospital environment. The most prominent example of this is to be found in the numerous publications dealing with the emergence of antibiotic-resistant staphylococci.[12] Hospital staphylococci tend to show a remarkable incidence of antibiotic-resistant strains and these in turn are characterized by belonging in great majority to phage Group III.[13] Our results would indicate that our hospital strains are further characterized by the common ability to produce beta toxin. Considering the great variation in the reported incidence of beta toxin production among strains surveyed,

it seems likely that this biotype may be peculiar to certain areas and the conflicting reports which have appeared[1] may be explicable on the basis of locale and origin of the strains.

Considerable interest may be attached to the observation that the lesion-producing staphylococci were significantly more toxigenic than carrier strains, and that the organisms carried by the hospital staff more closely resembled the organisms isolated from lesions, with respect to toxigenicity, than they resembled those carried by the normal population or even patient carriers.

Considering the large numbers of organisms which may be liberated into the environment from open active lesions, it would seem reasonable to assume that these lesions constitute an important primary source with respect to the initiation of hospital infections, an assumption strongly supported by the recent work of Barber and Burston.[14] Our demonstration of the toxigenicity of strains isolated from such lesions would imply that their importance resides not only in the probability of their attaining high concentration in the environment, but also their marked ability to establish themselves in tissue, as opposed to the lesser ability of normal carrier strains.

Hospital staff members also constitute an important group with respect to cross-infection. Not only are they potentially in close contact with susceptible patients but also many workers have demonstrated an increased carrier rate among those who work within the semiclosed community of the hospital, and our own hospital carriers have been shown to have a heavier nasal staphylococcal flora than normal nonhospital carriers. Further, it is well known that antibiotic-resistant variants tend to become established as common strains carried by the hospital staff and infections caused by these strains have, in recent years, given rise to a good deal of difficulty from a therapeutic point of view. Our demonstration of the rather high level of toxigenicity of hospital carrier strains adds another facet to their character, implying that these strains are fundamentally more dangerous then those carried by patients or visitors to the hospital.

The possible explanations of the predominance of toxigenic strains in our hospital carriers are of some interest. The factors involved in the institution and maintenance of the carrier state remain in the realm of speculation. The character of the strains isolated from carriers is, however, obviously influenced by the numbers and nature of the strains to which a population is exposed. It would seem likely that patients with discharging lesions add considerable numbers of highly toxigenic strains to the semiclosed hospital environment with the result that the nurses, who are constantly exposed, would on this ground alone be more likely to carry fully toxigenic types. The large number of staphylococci in the environment and the close and constant association of nurses with infection, together with the fairly rapid exchange of strains[15] within the hospital population, may be quite sufficient to account for the increased carrier rate noted in hospital personnel.

It is tempting to speculate that virulence, expressed as toxigenicity, may have a bearing on the ability of a strain to implant and maintain the carrier state. The large number of toxigenic strains isolated from the nurses and the

increased carrier rate frequently noted in this group may be due not only to the fact that toxigenic strains are more common in the hospital environment, but also to the possible increased ability of fully virulent strains to implant on the nasal mucosa and institute the carrier state.

If this thesis is entertained, it becomes important to distinguish between those factors concerned with the initiation of the carrier state and those concerned with its maintenance. Although it is conceivable that the production of large amounts of toxin may be associated with the ability of the organism to implant it does not seem likely that a high level of toxigenicity is compatible with the prolonged maintenance of a stable equilibrium between host and parasite. It is well known that carrier strains of *Hemophilus influenzae* and *Neisseria meningitidis* frequently lack the surface antigens associated with virulence. The decreasing infectiousness of streptococcal carriers with the passage of time is considered to be due to a process of antigenic degradation of the carried strain, with particular reference to the M substance, and a concomitant loss of virulence.[16, 17]

These observations would imply that prolonged residence of an organism in the throat of a carrier is associated with a degradation in virulence, and it may be that this degradation is necessary in order to maintain the asymptomatic carrier state. The high level of toxigenicity of strains isolated from the nurses may then reflect not only the high proportion of toxigenic strains in the environment, but also a rapid turnover of specific strains so that each biotype is resident in the throat of a carrier only for a relatively short period of time. This rather rapid turnover of carrier strains within a hospital staff has recently been demonstrated by Lepper and associates.[15] The considerably lower toxigenicity of strains isolated from the normal population may then be a reflection of a much lower rate of turnover of carried strains. Highly virulent strains could still be primarily implanted but prolonged maintenance of the carrier state may be associated with a decreasing virulence of the organism.

SUMMARY

1. The distribution of alpha, beta, and delta hemolysin, staphylokinase, and total number of diffusible antigens in 570 strains of coaglulase-positive staphylococci has been determined.

2. A modified plasma plate technique for the qualitative determination of staphylokinase production is described.

3. Strains isolated from furuncles and carbuncles were shown to produce more diffusible antigens, alpha and delta hemolysin, and staphylokinase than did strains isolated from wound infections. These strains in turn were more toxigenic than those isolated from patients' throats.

4. The number of strains producing beta toxin did not differ significantly in the above three groups.

5. Strains isolated from normal carriers were similar in character to the strains isolated from the patients' throats although somewhat less toxigenic.

6. Strains isolated from hospital carriers were shown to constitute a rather homogeneous, highly toxigenic group.

7. A beta toxin producing biotype was shown to be common in our hospital enviroment.

8. The relationship between toxigenicity and virulence, hospital infection, and the carrier state is discussed.

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DETERMINATION OF URINARY EXCRETION OF RADIOCOBALT-LABELED VITAMIN B_{12} BY COBALT SULFIDE PRECIPITATION

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RADIOCOBALT-LABELED vitamin B_{12} has considerably simplified the diagnosis of various vitamin B_{12} malabsorption syndromes. It has led to the development of methods by which the absorption and subsequent utilization of vitamin B_{12} can be followed in health and disease.[1]

In the Schilling test, the use of radioactive vitamin B_{12} is of value in differentiating pernicious anemia with defective vitamin B_{12} absorption due to intrinsic factor deficiency from other macrocytic anemias in which a vitamin B_{12} absorption defect is not correctable by intrinsic factor. This test is especially useful in determining the presence or absence of pernicious anemia in patients who have been treated so as to obscure the hematological or clinical picture.

In principle, the Schilling test involves ingestion of a small tracer dose of radioactive vitamin B_{12} followed by intramuscular injection of a relatively large amount (1,000 mcg.) of nonradioactive vitamin B_{12} to raise the blood level of vitamin B_{12} above the renal threshold and force its urinary excretion. The extent of the urinary excretion of radioactivity thus becomes a reflection of the intestinal absorption of the tracer vitamin B_{12} dose. It is low in pernicious anemia and in total and partial gastrectomies, cases in which the elaboration of the intrinsic factor is deficient or absent, and is normal where the intrinsic factor is unaffected.[2, 3]

In doubtful cases or for confirmation of a diagnosis of pernicious anemia, the test can be repeated with the modification of giving the intrinsic factor orally along with the radioactive vitamin B_{12} tracer dose. With the deficient intrinsic factor now externally supplied, the typical patient with pernicious anemia will show an appreciably increased absorption of vitamin B_{12}.[2]

Currently, the usual method for determining the radioactivity of the urine in the Schilling test is to measure with a scintillation detector the radioactivity of large volumes of urine. This procedure suffers from the following disadvantages: (1) the counting efficiency of such large volumes is very low and, therefore, necessitates the ingestion by the patient of an increased dose of radioactive vitamin B_{12}; (2) the radioactivity determination is not specific for Co^{60} and, therefore, may be unsuited for use with patients who have received previous radioisotope administration.

These shortcomings prompted us to develop the more specific and sensitive

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method of utilizing the precipitation of radioactive cobalt originating from the Co-labeled vitamin B_{12} as the quite insoluble CoS and measuring its radioactivity in a small tube in a well-type scintillation counter.

METHODS

A tracer dose of 0.05 μc of Co^{60}-labeled vitamin B_{12}* (specific activity 1 μc per 1 μg) was mixed with 90 ml. of water and given orally to the fasting patient. One hour later 1,000 μg of nonradioactive vitamin B_{12} was injected intramuscularly. The patient was instructed to abstain from eating meat during the following 24 hours.

The patient's 24-hour urine was collected, its volume measured, and the cobalt sulfide precipitation carried out on 1,000 ml. placed in a 2 liter glass-stoppered Pyrex bottle. If the volume of the 24-hour urine was less than 1 liter, it was diluted with water to that volume.

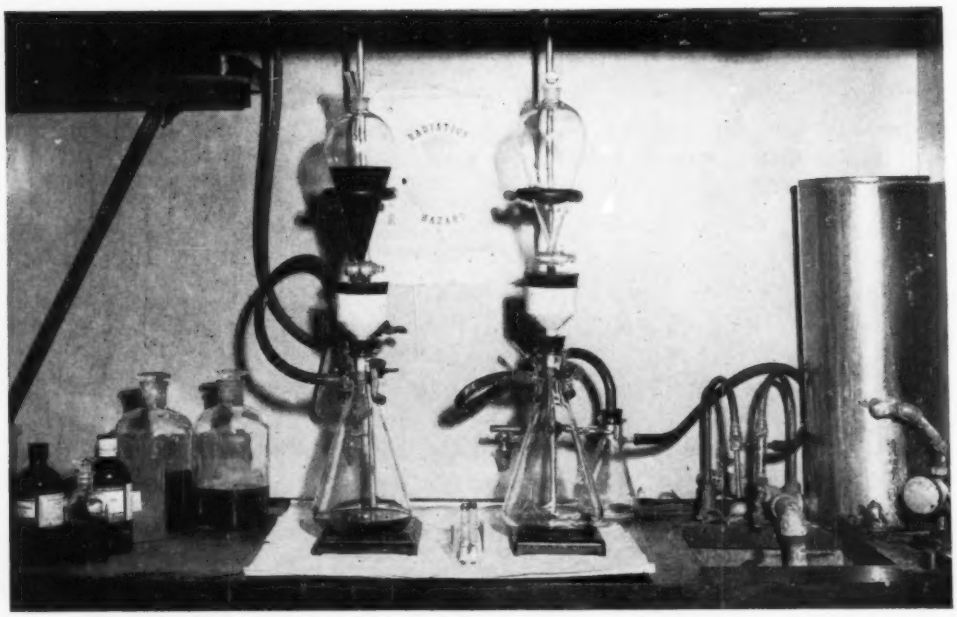

Fig. 1.—Vacuum filtration assemblies for filtering radioactive cobalt sulfide precipitated from patients' urines.

The entire cobalt sulfide precipitation procedure was performed in the hood. Four milliliters of 1 N cobaltous chloride was added to the urine as carrier for the small amount of excreted urinary cobalt, and 300 Gm. of NH_4Cl was dissolved in the urine with slight heating to facilitate solution. The addition of NH_4Cl prevents the subsequent formation of colloidal cobalt sulfide, which usually occurs when cobalt is precipitated as the sulfide from the urine. The amount of NH_4Cl which prevents the formation of the colloid differs with the composition of the urine, but the addition of 300 Gm. of NH_4Cl has proved to be sufficient for all urines we have encountered so far. Five milliliters of *n*-octyl alcohol were then added to reduce foaming. The mixture was heated just to boiling, and 6 ml. of 8.2 per cent $(NH_4)_2S$ (Fisher reagent) was added. After cooling, the black cobalt sulfide precipitate was isolated by vacuum filtration.

For filtration, we have found it convenient to transfer the mixture in several portions to a 1 liter separatory funnel, whose shortened stem was inserted through a center hole in a No. 13 rubber stopper. The rubber stopper, in turn, fitted tightly in a Coors No. 2 Buchner funnel, whose bottom plate was covered with two thicknesses of 7.0 cm. glass

*Source: Merck & Company, Inc., Rahway, N. J.

fiber filter paper.* The whole assembly was mounted on a 2 liter filtration flask, as shown in Fig. 1. A battery of several filtration assemblies enables the technician to carry out a number of determinations at the same time.

After filtration, the filter paper with the cobalt sulfide precipitate was carefully removed, transferred to a small Lusteroid tube,† and packed into a volume of 2 ml. The glass fiber filter paper has the advantage of having high retention qualities and unusual pliability. The cobalt sulfide is then counted in a well-type scintillation counter against the dose standard. The standard is prepared by diluting 0.05 μc of Co^{60} vitamin B_{12} activity with water to 100 ml. in a volumetric flask. Three milliliters of the diluted solution is counted in a scintillation well counter in a plastic test tube.

Each patient's urine was submitted to two precipitations. The second precipitation procedure differed from the first in that the addition of NH_4Cl and *n*-octyl alcohol and the heating of the urine prior to the addition of $(NH_4)_2S$ were omitted.

When low urinary Co^{60} recovery indicated a repetition of the procedure with intrinsic factor, 25 mg. of B_{12}-free intrinsic factor‡ mixed with the tracer dose of vitamin B_{12} was given orally to the patient. The repeat procedure may be started after a 3-day interval.

Recovery studies, in which a known amount of Co^{60}-labeled vitamin B_{12} was added to an assortment of different urines, showed an average radioactivity recovery of 94 ± 5 per cent in the combined cobalt sulfide obtained from two successive precipitations. A series of duplicate determinations on urines of patients who had received the vitamin B_{12} absorption test indicated that the reproducibility of this method falls within the above range of analytic accuracy.

RESULTS

Employing the methods described and using a 2″ × 2″ NaI (Tl) scintillation crystal well counter, each one per cent of activity recovered from urine registered 270 c.p.m. over background, or more than twice the background count. In instances of normal recovery, where averages approximate 18 per cent of the administered dose, the activity recovered from a 0.05 μc dosage cannot be adequately detected by the usual methods. Counting the radioactivity of 1,000 ml. of urine from normal subjects in direct contact with a 1½ by 1 inch NaI scintillation probe counter, or measuring it in three equal portions in the well counter directly over a 2″ × 2″ NaI crystal, gives extremely poor statistics of counting, since the counting rate is only slightly higher than background. The precipitation method results in about 400 times greater counting efficiency than with whole urine using the above scintillation probe, while it is 16 times more efficient than counting 333 ml. samples directly over the well counter.

The urinary recovery by the precipitation method, using 0.05 μc of Co^{60} activity in 0.05 μg of vitamin B_{12} was determined in 16 normal patients, 4 cases of pernicious anemia, and 3 patients with absorptive defects. The results are presented in Tables I, II, and III.

In one normal subject the percentage urinary recovery with 0.05 μc of activity was 26.6 per cent. With the Schilling technique, using 0.5 μc of activity labeled to 0.5 μc of vitamin B_{12}, the same patient showed 26.9 per cent urinary recovery.

The presence of massive albuminuria did not seriously interfere with the precipitation test. The procedure was attempted in one nephritic patient, whose total 24-hour urinary volume was 1,160 ml., with a protein concentration of 0.63 Gm. per cent. The urine was heated to boiling and was then filtered with

*Source: Fisher Scientific, Pittsburgh, Pa.
†Source: Lusteroid Corporation, Maplewood, N. J.
‡Source: The Armour Laboratories, Kankakee, Ill.

vacuum prior to the cobalt sulfide precipitation to remove most of the albumin. Fourteen per cent of the administered dose was recovered in the supernatant, while 1.17 per cent of the administered activity was precipitated with the protein. The activity in the protein may well have been due to trapping of Co^{60} by the voluminous precipitate. It is apparent that urinary Co^{60}-vitamin B_{12} activity was not significantly protein-bound in the massive albuminuria of the patient tested.

TABLE I. PERCENTAGE RECOVERY OF Co^{60}-VITAMIN B_{12} IN THE URINE OF 16 NORMAL PATIENTS, USING THE COBALT SULFIDE PRECIPITATION METHOD

PATIENT	PER CENT RECOVERED	PATIENT	PER CENT RECOVERED
1. G. A.	18.5	9. J. M.	21.4
2. L. C.	14.6	10. G. Q.	13.9
3. J. B.	17.7	11. J. R.	15.0
4. A. D.	14.6	12. G. R.	16.7
5. W. H.	17.7	13. J S.	22.2
6. C. J.	19.0	14. W. D.	17.0
7. L. B.	27.0	15. S. V.	23.4
8. L. C.	23.0	16. L. W.	11.5
Range of recovery:	11.5-27.0		
Average recovery:	18.3		

TABLE II. PERCENTAGE RECOVERY OF Co^{60}-VITAMIN B_{12} IN THE URINE OF 4 PATIENTS WITH PERNICIOUS ANEMIA USING THE COBALT SULFIDE PRECIPITATION METHOD

PATIENT	PER CENT RECOVERY	
	WITHOUT INTRINSIC FACTOR	WITH INTRINSIC FACTOR
1. B. A.	1.0	29.2
2. E. L.	2.2	14.2
3. A. D.	1.4	10.0
4. H. Z.	0.04	21.5
Range of recovery:	0.04-2.2	10.0-29.2
Average recovery:	1.2	18.8

TABLE III. PERCENTAGE RECOVERY OF Co^{60}-VITAMIN B_{12} IN THE URINE OF 3 PATIENTS WITH MEGALOBLASTIC ANEMIA AND ABSORPTIVE DEFECTS, USING THE COBALT SULFIDE PRECIPITATION METHOD

PATIENT	PER CENT RECOVERY	
	WITHOUT INTRINSIC FACTOR	WITH INTRINSIC FACTOR
1. F. L.	8.1	11.3
2. E. C.	2.5	4.6
3. G. F.	9.8	3.3

When the cobalt sulfide precipitation procedure was applied to aliquots of urines from patients whose only radioisotope administration consisted of I^{131}, the radioactivity admixed with the precipitated cobalt sulfide (after washing it in the Buchner funnel with 200 ml. of aqueous 30 per cent NH_4Cl) was on the average only 1.3 per cent of the corresponding urine aliquots evaporated almost to dryness. The cobalt sulfide precipitation method renders it possible, therefore, to use the vitamin B_{12} absorption test on patients who had recently received radioactive iodine. Depending on the I^{131} content of the patient's urine, a dosage ranging from 0.05 μc to 0.5 μc of Co^{60}-labeled vitamin B_{12} is recommended. The Co^{60} urinary excretion of 9 patients who, for thyroid study, had received 50 μc of I^{131} in the preceding week was successfully determined by this method.

DISCUSSION

The increased counting efficiency of the cobalt sulfide method, as compared with other available techniques, makes possible the repeated use of the test with minimal exposure of the patient to Co^{60}, which has a physical half-life of 5.3 years. The flushing dose further contributes to a reduction in the biologic half-life. The use of 0.05 μc of Co^{60} activity allows for statistically accurate counting in the method described and could be easily reduced to even one half of this amount. Repeated testing may well be a means of assaying intrinsic factor activity in those patients with incipient intrinsic factor defects.

Urinary recovery of Co^{60} activity, using the 0.05 μc per 0.05 mg. dose of labeled vitamin B_{12}, is of the same order as reported with larger doses.[4-7] Less than 5 per cent recovery suggests unequivocal absorptive defects; 5 to 10 per cent recovery is equivocal and should be an indication for repeating the test with intrinsic factor. In the 16 normal subjects tested, recovery of less than 11.5 per cent of the administered dose was not observed.

It is not unusual that candidates for the Schilling test have received radioiodine for thyroid function study because of the superficial clinical resemblance between myxedema and pernicious anemia. The chemical separation of Co^{60} from I^{131} by the precipitation method obviates a long waiting period in many instances.

SUMMARY

A method is described for the determination of urinary excretion of radiocobalt-labeled vitamin B_{12} in the Schilling test by cobalt sulfide precipitation. The results obtained for normal and pathologic groups of patients were similar to those using the higher conventional dosage of radioactive vitamin B_{12}. This procedure, however, has a twofold advantage: (1) Its high counting efficiency permits the use of only a 0.05 μc dose of Co^{60}-vitamin B_{12} with a high counting rate of 2,700 c.p.m. for a 10 per cent recovery using a $2'' \times 2''$ sodium iodide crystal. (2) Its relative specificity makes it possible to administer the Schilling test to patients who have previously been given other radioisotopes and are still excreting radioactivity not precipitable from urine by sulfide ions.

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THE DETERMINATION OF INULIN IN BLOOD AND URINE USING GLUCOSE OXIDASE FOR THE REMOVAL OF INTERFERING GLUCOSE

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IN MOST presently available methods for the determination of inulin in blood and urine, glucose is an interfering substance during colorimetry. At normal levels of blood glucose this interference is small and one assumes that the "blood blank," which is due in part to blood glucose, remains constant and thus allows for adequate correction. At high and varying concentrations of glucose in blood and urine, however, such as are encountered during studies of renal glucose clearance, glucose interference becomes a major problem and prevents the accurate quantitation of inulin in blood and urine. It would be evidently desirable to eliminate glucose before the color reactions are carried out and, indeed, procedures to do so have been devised. Fermentation by starved preparations of bakers' yeast have been used by Smith and collaborators[1] and have again been recommended by White and Samson[2] while Little[3] recommends elimination of glucose in hot alkali. The glucose intereference can also be reduced by careful manipulation of the conditions used during the chromogenic reactions with resorcinol or anthrone.[4, 5] A simple enzymatic procedure for the removal of interfering glucose prior to the determination of inulin forms the basis for this report.

Glucose oxidase catalyzes the oxidation of glucose to gluconic acid which produces no color with resorcinol. However, considerable difficulties were encountered when crude, commercially available glucose oxidase preparations were used. As is the case with yeast the blank values were high and unpredictable, an observation which can probably be imputed to the high polysaccharide content of these crude preparations. Purified preparations of glucose oxidase were then made available to the authors through the courtesy of Dr. V. Auerbach, from the Department of Biological Chemistry, Harvard Medical School, and Dr. L. A. Underkofler of the Takamine Laboratories, Clifton, New Jersey. The purification method used by Dr. Auerbach will be published in

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detail[6] and a purified preparation is to be made commercially available. These two preparations did not give rise to significant blank values in the color reaction. After the enzymatic removal of glucose, inulin determinations were carried out according to Higashi's modification[7] of the method of Roe.[4] Deproteinization, however, was carried out according to Somogyi,[8] utilizing zinc sulfate and sodium hydroxide.

MATERIALS AND METHODS

Reagents.—The two purified glucose oxidase preparations used contained approximately 34.000 glucose oxidase units per gram of enzyme. The amount of catalase and glucomutarotase present in these two preparations has not been measured. In a volume of 3 ml. one-half gram of either preparation completely oxidized 15 mg. of glucose during a 60-minute incubation period at 37° C. It is evident that each new batch of enzyme has to be evaluated for its potency under the conditions used. The enzyme solution was prepared by dissolving 0.25 mg. of enzyme per ml. of 0.154 M acetate buffer, pH 5.6. The enzyme solution can be frozen and stored without any detectable loss of activity for several weeks.

In addition the following reagents were used:

Acetate buffer, pH 5.6 (19 parts 0.154 M acetic acid and 181 parts 0.154 M sodium acetate).

Zinc sulfate, 5 per cent in water.

Sodium hydroxide, 0.5 N.

Resorcinol, 0.15 per cent in 95 per cent ethanol.

Concentrated hydrochloric acid with 7.5 mg. ferric chloride per liter.

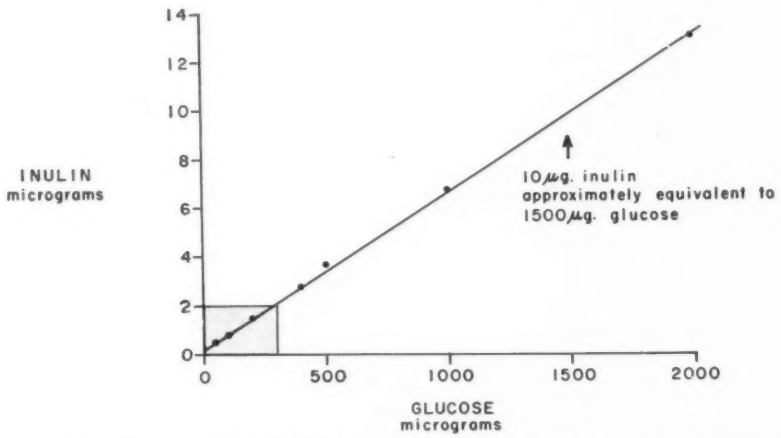

Fig. 1.—Relative chromogenic activity of glucose and inulin in the resorcinol reaction (20 minutes at 80°C.).

Procedure.—One milliliter of each blood or urine sample and 2 ml. of enzyme solution are carefully pipetted into 10 ml. test tubes; a drop of caprylic alcohol is added to each tube to prevent foaming during the subsequent aeration. The test tubes are placed in a test tube rack and incubated in a water bath at 37° C. for 60 minutes. Mixing and oxygenation is insured by the slow delivery of oxygen or air, using a glass manifold fitted with rubber tubing into which are inserted number 25 hypodermic needles. Polyethylene tubing is threaded over the needles in appropriate length to reach the bottom of each tube. Deproteinization is then carried out by adding 1 ml. of zinc sulfate and 1 ml. of sodium hydroxide to each tube. After thorough mixing the tubes are centrifuged and an aliquot of the supernatant is appropriately diluted for the subsequent reaction with resorcinol, according to Higashi and Peters.[7] The incubation procedure is similar to that previously reported in

more detail for the determination of glucose in blood and urine.[9] It should be emphasized at this point that great care is necessary in the pipetting of all samples to insure adequate results.

DISCUSSION

Fig. 1 illustrates the quantitative aspects of the interference by glucose in the resorcinol color reaction. In this instance 1.5 mg. of glucose was found to be equivalent to 10 μg of inulin or fructose. A number of similar experiments have been carried out and, in our hands, the color produced by glucose was on the average 150 to 200 times less than that produced by the same amount of inulin. The shaded area in the left lower corner indicates the range of interference which is encountered in glucose titration studies. Thus, at blood glucose concentrations of 750 mg. per cent and concomitant plasma inulin levels of 25 mg. per cent, the error in the inulin measurement is approximately 20 per cent. After incubation with glucose oxidase no interference can be detected.

TABLE I. RECOVERY OF INULIN FROM PLASMA AFTER INCUBATION WITH GLUCOSE OXIDASE FOR 1 HOUR

INULIN ADDED (μG/ML.)	INULIN RECOVERED (μG/ML.)	RECOVERY (%)
0.005	0.0053	106
0.010	0.0099	99
0.020	0.0204	102
0.025	0.0245	98

TABLE II. INULIN DETERMINATION IN PLASMA CONTAINING DIFFERENT AMOUNTS OF GLUCOSE, WITH AND WITHOUT PREVIOUS INCUBATION WITH GLUCOSE OXIDASE

GLUCOSE (MG./ML.)	INULIN ADDED (MG./ML.)	INULIN MEASURED WITHOUT INCUBATION WITH GLUCOSE OXIDASE (MG./ML.)	INULIN MEASURED AFTER INCUBATION WITH GLUCOSE OXIDASE (MG./ML.)
0.4	0.0200	0.0220	0.0206
0.8	0.0200	0.0242	0.0204
1.0	0.0200	0.0262	0.0201
2.0	0.0200	0.0330	0.0194

Table I illustrates the recovery of inulin added to plasma. Recoveries varied between 98 and 106 per cent. In another experiment, increasing quantities of glucose were added to plasma containing a known amount of inulin, and inulin determinations were carried out before and after incubation with glucose oxidase. As shown in Table II the amounts of inulin measured after treatment with glucose oxidase correlated well with the amounts added, whereas an increasing error was introduced by increasing glucose concentration when the samples were not pretreated with glucose oxidase. In addition the plasma blank was determined in 26 samples obtained from 16 fasted subjects. The mean blank was found to be 0.75 mg. per cent and the range 0 to 3.25 mg. per cent. The method described has successfully been applied to a large number of glucose titration studies which will be reported.[10]

SUMMARY

A method for the determination of inulin in the presence of high concentrations of glucose is described, making use of the property of glucose oxidase to catalyze the oxidation of glucose to gluconic acid, a substance which does not interfere in the resorcinol reaction. No appreciable enzyme blank values were encountered when purified preparations of glucose oxidase were used. Recovery experiments and data on the plasma blank obtained with this method are reported.

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PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

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ABSTRACTS

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63. THE NATURE OF THE COAGULATION DEFECT IN PSEUDOHEMOPHILIA B

SHIRLEY A. JOHNSON, PH.D. (BY INVITATION), RAYMOND W. MONTO, M.D., AND JOHN W. REBUCK, M.D., DETROIT, MICH.

Pseudohemophilia B (Quick), thrombocytopathy, is a mild to moderate hemorrhagic disease showing a familial tendency occurring in either sex and characterized by a prolonged bleeding time and poor prothrombin consumption. The mechanism of the abnormal bleeding has been postulated to be on the basis of a vascular defect, platelet functional abnormality, or lack of a plasma factor.

Prothrombin consumption was found to be poor (below 20 sec.) in 10 patients suffering from pseudohemophilia B. When an assay for platelet factor III activity (Alkjaersig, Abe, and Seegers) was carried out very little was found in platelets of these patients as compared with platelets of normal individuals. The addition in vitro of purified platelet factor III of bovine origin corrected the prothrombin consumption of patients with pseudohemophilia B.

Cortisone and prednisone therapy did not alter the prothrombin consumption in pseudohemophilia B. Investigation is underway to prepare platelet factor III of bovine origin for intravenous use in patients with pseudohemophilia B and to observe the ultramicroscopic morphology of the platelets utilizing the electron microscope. The administration of platelet factor III to thrombocytopenic patients resulted in a marked increase in vascular resistance. Assay for platelet factor III in relatives of patients with pseudohemophilia B may afford an opportunity to establish the genetics of this hemorrhagic disorder.

These preliminary studies suggest that pseudohemophilia B is a hemorrhagic disease resulting from a deficiency of platelet factor III.

64. THE EFFECTS OF ACUTE REDUCTION OF RENAL ARTERY BLOOD PRESSURE ON RENAL HEMODYNAMICS AND EXCRETION OF ELECTROLYTES AND WATER

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(INTRODUCED BY CHARLES GRUBER, M.D.)

In the present investigation, renal responses to acute reduction in renal artery pressures were measured in patients during resection of thoracic aortic aneurysms. During occlusion of the thoracic aorta, lasting from 47 to 64 minutes, the blood flow and mean arterial pressure of the distal aortic segment were maintained and controlled by a mechanical pump which shunted blood from the brachial to the femoral artery, thus perfusing the distal aortic segment in a retrograde manner. By-pass blood flow was varied by altering either the size of the pumping chamber or the rate of blood shunted through the system.

During the by-pass, the mean arterial pressure in the distal aortic segment ranged from 35 to 75 mm. Hg. At these levels of mean perfusion pressure the renal plasma flow (PAH) fell to from 7 to 33 per cent, and the glomerular filtration rate (inulin) fell to from 9 to 38 per cent, of the preocclusion level; this change occurred within 9 to 26 minutes. There was a concomitant reduction in urine formation and sodium excretion during this same period.

No urine was formed after the first 25 to 33 minutes of reduced blood flow. With re-establishment of flow and pressure to preocclusion conditions, there was a rapid return toward the control level of these renal functions.

In one patient with a left atrial-femoral artery shunt, the mean pressure ranged from 60 to 120 mm. Hg with a pulse pressure of approximately 35 mm. Hg. During the entire period, this patient continued to form urine and to maintain measurable renal function.

It is concluded from these observations that:

1. With the reduction of mean renal arterial pressure to from 35 to 65 mm. Hg, accompanied by a low pulse pressure, urine formation is not observed.

2. However, with mean arterial pressures of 60 to 65 mm. Hg, if the pulse pressure is increased, there may be continued production of urine.

3. Finally, despite these subfiltration pressures, the kidney is apparently protected from the effects of ischemia as evidenced by the rapid postocclusion return of functions toward normal.

65. SUPPRESSION OF INFLUENZA VIRUS PNEUMONIA WITH RNA

EDWIN D. KILBOURNE, M.D., NEW YORK, N. Y.

During investigation of the mechanisms of corticosteroid action in influenza virus infection, it was discovered that the administration of sodium ribonucleate (RNA) to infected mice induced a surprising suppressive effect upon the appearance of pulmonary lesions and death. Antagonism of the enhancing effect of cortisone on virus yields was not demonstrated.

From 7 experiments with the mouse-adapted CAM strain of influenza A virus employing 5 to 20 mice per group, the following observations have been derived. The subcutaneous administration of 50 mg. daily of RNA to 18 to 20 gram CFW mice has in every experiment delayed the appearance of grossly evident pulmonary lesions in mice infected intranasally with 10^6 to 10^8 EID_{50} of CAM virus. Reduction in percentile lesion scores was most striking in mice

which received the larger viral inocula. This observation is probably related to the sharper end points and greater uniformity characteristic of infections of high multiplicity.

Measurement of hemagglutinating and infective virus in lungs from these experiments has not revealed significant differences in virus concentrations of lungs from control and RNA-treated mice. The specificity of the RNA lesion-sparing effect in relation to host or virus RNA metabolism remains to be determined. However, in context with other studies from this laboratory (to be reported separately) a possible relation of the lesion-sparing effect of exogenous RNA to the synthesis of influenza virus (an RNA virus) is suggested. These corollary studies have demonstrated enhanced yields of influenza viruses in eggs injected with RNA.

It is tentatively proposed that the administration of exogenous RNA may "spare" the endogenous host RNA deviated by the virus to its own synthesis, and that the sparing effect reduces host cell damage in the presence of unaltered viral synthesis.

66. RADIOACTIVE TRIOLEIN EXCRETION AND INCREASED INTESTINAL MOTILITY

ARTHUR P. KLOTZ, M.D., ROBERT O. MOSSER, M.D. (BY INVITATION), AND LUDWALD O. P. PERRY, M.D. (BY INVITATION), KANSAS CITY, KAN.

Standardization of normal limits for fecal excretion of radioactive triolein has provided a simple and rapid clinical method for the determination of increased fat in the stool. Isotope studies in patients with diabetes have suggested that the steatorrhea of diabetic nocturnal diarrhea may be on the basis of altered motility since fat absorption was found to be normal during a remission in one such patient.

Alteration of fat absorption can be demonstrated by increasing intestinal motility in otherwise normal subjects with the use of castor oil. Patterns of excretion were studied for varied time intervals of purgation after tracers were administered. Results show fat excretion to be well above normal limits.

The clinical importance of such information relates to the common problem of functional diarrhea with accompanying increased small bowel motility and its possible confusion with other causes of steatorrhea when the radioactive triolein test is used as a screening procedure.

67. IN VITRO METABOLISM OF TRIIODOTHYRONINE BY VARIOUS ORGANS

F. C. LARSON, M.D., K. TOMITA, PH.D. (BY INVITATION), AND E. C. ALBRIGHT, M.D., MADISON, WIS.

Previous studies have shown that rat kidney slices contain an enzyme which deiodinates thyroxine to yield triiodothyronine. Further, a soluble enzyme has been obtained from sonically disintegrated rat kidney mitochondria which converts thyroxine and triiodothyronine to the corresponding acetic acid analogues.

In the present study the metabolism of I^{131}-labeled triiodothyronine by mitochondria of rat liver, heart, and brain was examined. Mitochondria of

kidney were used as a control. Both intact mitochondria and a soluble enzyme prepared by dialysis of sonic disintegrated mitochondria from each organ studied were employed. The reaction mixture containing phosphate buffer (pH 7.4), DPN, triiodothyronine and enzyme preparation was incubated at 37° C. for 2 hours. Following incubation the reaction mixture was applied directly to a paper strip and chromatographed with tertiary amyl alcohol—2 N ammonia solvent system. Localization of radioactivity was accomplished with a chromatograph scanner, while the position of unlabeled carrier was determined by means of the diazo reaction.

Two separate reactions were observed: (1) deiodination and (2) conversion of triiodothyronine to triiodothyroacetic acid.

Intact mitochondria from the kidney and liver were found to actively deiodinate triiodothyronine. Intact mitochondria from the heart exhibited very weak deiodinating activity, and those from the brain demonstrated none. Sonic disintegration of mitochondria completely abolished their deiodinating activity.

Conversion of triiodothyronine to triiodothyroacetic acid was observed with intact liver mitochondria. The enzyme activity was comparable to that of kidney, and like kidney was retained after solubilization of the mitochondria. Heart mitochondria were weakly active in conversion of triiodothyronine to triiodothyroacetic acid, and those from brain were inactive.

68. CORRECTION OF ANDROGEN DEFICIENCY IN KLINEFELTER'S SYNDROME BY CHORIONIC GONADOTROPHIN TREATMENT

ROBERT B. LEACH, M.D. (BY INVITATION), WILLIAM O. MADDOCK, M.D., C. ALVIN PAULSEN, M.D. (BY INVITATION), JOHN LANMAN, M.D. (BY INVITATION), DETROIT, MICH., AND WARREN O. NELSON, M.D. (BY INVITATION), NEW YORK, N. Y.

Testicular biopsy and urinary gonadotrophin assays permit the separation of eunuchoidism into two main types: *hypogonadotrophic,* characterized by infantile testes and low gonadotrophin excretion; and *hypergonadotrophic,* characterized by damaged or defective testes and high gonadotrophin levels. A therapeutic trial of chorionic gonadotrophin has been suggested as a clinical means of separation, a positive response being expected in hypo- but not in hypergonadotrophic eunuchoids.

In Klinefelter's syndrome, a type of hypergonadotrophic hypogonadism of congenital origin, Leydig cell deficiency may or may not be present; all degrees of androgen deficiency are encountered between the extremes of normal sexual development and marked eunuchoidism. In even the most eunuchoidal cases, however, testicular biopsy usually reveals relatively abundant Leydig cells of surprisingly good appearance. In an attempt to increase androgen production by these cells, chorionic gonadotrophin 5,000 units 3 times weekly intramuscularly was administered for 2 weeks to 11 months to 15 patients having Klinefelter's syndrome. The patients' ages ranged from 15 to 51, and averaged 29 years. The diagnosis was based in each case on the finding of elevated urinary gonadotrophin excretion, small testes exhibiting the typical biopsy pattern of clumped Leydig cells interspersed among hyalinized seminiferous

tubules, and buccal smears or skin biopsies showing a female chromatin pattern. Eight patients were moderately eunuchoidal, 3 were slightly eunuchoidal, and 4 showed normal adult sexual development.

During treatment, 10 patients reported enhanced libido and increased number of erections. Growth of genitalia was noted in 7 of the eunuchoidal patients. Urinary estrogen excretion, which has proved a sensitive criterion of Leydig cell response to stimulatory therapy in normal men and hypogonadotrophic eunuchoids, was studied in 10 patients. In each, levels increased to at least twice pretreatment values. Since symptomatic improvement promptly disappeared upon stopping treatment, chorionic gonadotrophin offered no advantage over testosterone therapy.

It is concluded that the Leydig cells in Klinefelter's syndrome, despite elevated endogenous gonadotrophin levels, remain responsive to administered gonadotrophin. Consequently, a therapeutic trial of chorionic gonadotrophin will not differentiate hypo- from hypergonadotrophic syndromes.

69. THE EXPERIMENTAL PRODUCTION OF SPLENIC SEQUESTRATION OF ERYTHROCYTES IN PATIENTS WITH SICKLE CELL TRAIT

WILLIAM C. LEVIN, M.D., WILLIAM D. BAIRD, M.D. (BY INVITATION), JOHN E. PERRY, M.D. (BY INVITATION), AND WILLIAM W. K. ZUNG, M.S. (BY INVITATION), GALVESTON, TEXAS

Autologous erythrocytes labeled with $Na_2Cr^{51}O_4$ were transfused during periods of hypoxia (duration 60 to 90 minutes) induced by the breathing of mixtures of oxygen and nitrogen. Radioactivity over the spleen, liver, and heart was determined by the external counting method of Jandl and associates and erythrocyte survival time was determined by a modification of the method of Read and associates.

Hypoxia simulating the oxygen content of the atmosphere at 7,000 and 8,000 feet altitude produced no changes in these parameters in normal humans. Similarly, no changes were observed in one patient with sickle cell trait (hemoglobin AS) who was exposed to hypoxia simulating an altitude of 5,000 feet. Two patients (hemoglobin AS) made hypoxic to simulate an altitude of 7,000 feet and two patients (hemoglobin AS) made hypoxic to simulate an altitude of 8,000 feet behaved alike. In each instance there was an increase in splenic activity and/or a reduction in hepatic activity which persisted for 3 to 15 days following the period of hypoxia. Thereafter, normal splenic and hepatic activity was established. The duration of splenic hypersequestration appeared to be directly related to the degree of hypoxia. During the period of splenic concentration of labeled erythrocytes, erythrocyte survival time was significantly reduced ($T\frac{1}{2}$—13 to 20 days), but after spleen and liver activity returned to normal, normal erythrocyte survival time was established. One patient (hemoglobin AA) whose hematologic characteristics suggested a diagnosis of thalassemia minima behaved in a similar fashion following exposure to hypoxia simulating an altitude of 8,000 feet.

These data indicate that induced hypoxia may produce transient splenic hypersequestration of labeled erythrocytes containing S hemoglobin. A similar phenomenon is suggested in the presence of thalassemia.

70. DIFFUSING CAPACITY OF PULMONARY MEMBRANE AND PULMONARY CAPILLARY BLOOD VOLUME IN NORMAL SUBJECTS; THE EFFECTS OF EXERCISE AND BODY POSITION; PRELIMINARY OBSERVATIONS IN CARDIAC PATIENTS

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A measurement of over-all diffusing capacity of the lungs by the single breath carbon monoxide technique depends on the thickness and area of the pulmonary membrane, the volume of blood in the capillaries, and the rate at which this blood reacts with CO. In congestive heart failure, increased thickness of the membrane and increased capillary volume might coexist; since they influence over-all diffusing capacity in opposite directions, a change in one might mask a simultaneous change in the other. To separate these two factors, three measurements of total diffusing capacity are made at three different oxygen tensions. Reaction rate of CO with hemoglobin is known approximately from in vitro experiments; its reciprocal, the resistance to diffusion due to reaction rate, increases linearly with oxygen tension. If the reciprocal of over-all diffusing capacity, which is the total resistance to diffusion, is plotted against reaction rate resistance, membrane diffusing capacity and capillary volume may then be estimated (Forster and associates: Am. J. Physiol. **183**: 615, 1955). Twenty-two such measurements were made in 18 seated normal subjects. Average capillary volume was 89.0 ml., and average membrane diffusing capacity was 56.5 ml./mm. Hg/min. Capillary volume and membrane diffusing capacity are positively correlated with height, weight, body surface area, and vital capacity. Exercise in 4 normal subjects caused an increase of 15.9 ml. in capillary volume and 16.8 ml./mm. Hg/min. in membrane diffusing capacity. The recumbent position produced a mean increase in capillary volume of 26.8 ml. in 4 normal subjects, but did not change membrane diffusing capacity consistently. Preliminary studies in cardiac patients suggest the potential usefulness of estimating separately capillary volume and membrane diffusing capacity. Two patients with untreated congestive heart failure showed an increased capillary volume, but a decreased membrane diffusing capacity; both values changed toward normal with treatment. One patient with mitral insufficiency, not in failure, showed an increased capillary volume but a normal membrane diffusing capacity. Two of these patients showed a normal over-all diffusing capacity while breathing room air.

71. OBSERVATIONS ON THE ROLE OF THE PLASMA ERYTHROPOIETIC FACTORS IN POLYCYTHEMIA VERA

JAMES W. LINMAN, M.D. (BY INVITATION), AND FRANK H. BETHELL, M.D., CHICAGO, ILL. AND ANN ARBOR, MICH.

The mechanisms governing erythropoiesis include humoral factors which exert important physiologic and pathophysiologic influences over the production of erythrocytes. Current information suggests the existence of two such factors. One is thermostable, ether soluble, and when administered to normal rats stimulates erythroblastic cellular division. This is manifested by erythrocytosis

due to microcytes with decreased osmotic resistance, reticulocytosis, and increased numbers of marrow erythrocytic precursors. The other factor is relatively thermolabile, insoluble in ether, and augments hemoglobin synthesis.

The overproduction of erythropoietic factors in experimental situations and in human subjects with secondary polycythemia and some types of anemia is most likely related to general or local tissue hypoxia. Patients with polycythemia vera also have enhanced plasma erythropoietic activity. This finding, together with the absence of demonstrable hypoxia in polycythemia vera and the evidence previously reported against the production of the plasma factors by hemopoietic elements, suggested to us that the humoral factors may have etiologic significance in this disorder. Additional support for such a causal relationship has been obtained.

Microcytes with decreased resistance to lysis in hypotonic media similar to those present in rats injected with the thermostable plasma factor have been demonstrated in patients with polycythemia vera. Osmotic fragilities determined by a photocolorimetric method were not abnormal but decreased resistance of the microcytes was evident with a direct cell enumeration technique employing red cell pipettes and hypotonic salt solutions as diluents. To date we have studied two patients, both before therapy and after normal erythroid values were achieved following treatment with P^{32}. In both patients enhanced plasma erythropoietic activity persisted in spite of normal peripheral blood values as did the microcytes with abnormal osmotic behavior. Data from more patients in remission are being obtained.

On the basis of these preliminary observations, the plasma factors would appear to be concerned with the pathogenesis of polycythemia vera at least in some cases. The stimulus to the derangement in the erythropoietic regulatory mechanisms is unknown. Studies in regard to the presence of humoral factors concerned with excessive granulopoiesis and thrombopoiesis in polycythemia vera are in progress.

72. TREATMENT OF POLYCYTHEMIA VERA WITH MYLERAN

(1, 4-dimethylsulfonoxybutane)

PRELIMINARY REPORT

JOHN LOUIS, M.D., CHICAGO, ILL.

(INTRODUCED BY LOUIS R. LIMARZI, M.D.)

Ten patients with polycythemia vera were treated with Myleran. Ages ranged from 50 to 64 years; 3 were women. Duration of illness prior to the onset of Myleran therapy ranged from 3 to 8 years. The diagnosis was confirmed by usual hematologic techniques. All patients were previously treated with triethylene melamine, radioactive phosphorus, and intermittent venesection. Previous therapy was discontinued 1 to 12 months before initiation of Myleran therapy to facilitate re-establishment of the polycythemic state.

Four patients received Myleran therapy for less than 2 months, and 5 for longer than 7 months. In 7 patients the initial dose ranged from 4 to 10 mg. per day for 2 to 6 weeks. Three patients required venesection of 500 ml. each. One patient (hematocrit 70 per cent) required venesection of 4,500 ml. Initial hematocrits ranged from 48 to 70 per cent (6 patients >51 per cent) and leukocyte counts from 10,700 to 54,500 cells/c.mm. (5 patients $>12,500$

cells/c.mm.). The hematocrits and leukocyte counts became reduced in all patients during therapy. After the period of initial dosage the hematocrits ranged from 46 to 61 per cent (2 patients >51 per cent) and leukocyte counts ranged from 4,500 to 41,100/c.mm.[3] (3 patients >12,500 cells/c.mm.). Maintenance dosage varied from 4 mg. per day to 2 mg. per week for 4 to 9 months. To date maintenance therapy was not necessary in 3 patients (2 to 7 months). At present hematocrits range from 32 to 53 per cent (one patient >51 per cent) and leukocyte counts 3,900 to 26,200 cells/c.mm. (one patient >12,500 cells/c.mm.). Five patients had splenomegaly. The spleen is no longer palpable in 2, and is significantly reduced in 3. In the 3 patients with hepatomegaly, reduction in size could not be demonstrated

One patient developed marked anemia (hematocrit 25 per cent) leukopenia, (1,200 cells/c.mm.), and thrombocytopenia (30,000 platelets/c.mm.). The bone marrow, however, was hypercellular. The patient has since shown a progressive recovery of her peripheral blood elements.

Myleran appears to be effective in controlling the polycythemic state. Whether or not it will be generally useful in the treatment of polycythemia vera will depend on further clinical evaluation.

73. CORRELATION OF RADIOACTIVE (I^{131}) ROSE BENGAL TEST WITH LIVER FUNCTION TESTS

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The radioactive (I^{131}) rose bengal test is used as a measure of hepatic parenchymal function and biliary patency. In our series we have 48 cases serving as normal controls, 71 with cirrhosis, 53 with infectious hepatitis, 23 calculous patients, and 36 patients with carcinomatous extrahepatic obstruction. On the same day of testing, blood was drawn for the following chemistries: albumin/globulin ratio, alkaline phosphatase, icterus index, thymol turbidity, and gamma globulin turbidity. We have attempted to correlate these with the rose bengal uptake rates and blood counts.

Five microcuries of radioactive rose bengal solution was injected intravenously and a lead collimated scintillation counter placed over the liver. Uptake-excretion graphs were completed in 45 to 90 minutes and levels were again obtained in 6 and 24 hours. Blood samples of 1 ml. were drawn concurrently and measured in a deep-well counter.

Uptake rates were plotted against albumin/globulin ratios in the form of a scattergram. Most cases had an A/G ratio of less than 2:1. Ratios less than 1:1 had low uptakes and were predominantly cirrhotic. In all groups, the ratios were related more to the diagnosis and showed no significant correlation with uptake rates.

Alkaline phosphatase versus uptake likewise showed no significant correlation. As expected, carcinomatous and calculous obstruction had the highest alkaline phosphatase levels, but its uptake rates varied considerably.

Uptake plotted against icterus index showed good correlation. In all groups except for hepatitis, the cases with the lowest uptake had the highest icterus index and those with higher uptakes showed a lower icterus index. Hepatitis produced a higher uptake than the groups which had the same degree of icterus.

Uptake rates showed no significant relationship when compared with thymol turbidity and gamma globulin turbidity. There were wide variations in uptake rates at any given thymol or gamma globulin turbidity level.

Peak blood counts plotted against icterus index showed an excellent correlation. In all groups, it was evident that the higher the icterus, the higher were the peak blood counts.

The fact that the rose bengal (I^{131}) test is independent of the other liver function tests employed except for the icterus index would increase its sphere of usefulness.

74. ADAPTATION OF THE ANTIGLOBULIN TEST FOR USE WITH TANNIC ACID TREATED ERYTHROCYTES

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(INTRODUCED BY JOHN M. SHELDON, M.D.)

Although it is tempting to use the antiglobulin (Coombs') test in the study of antibodies which fail to give direct hemagglutination, results of this test are not meaningful when tannic acid treated erythrocytes are used (Boyden's technique), since these cells nonspecifically adsorb normal serum globulins. Attempts to exhaust tanned red blood cells' ability nonspecifically to take up globulin by preliminary incubation with several proteins were unsuccessful, but employing serum from a suitable species for this purpose has proved useful. The procedure is as follows: (1) tanned R.B.C. plus (2) antigen, (3) followed by preliminary incubation with serum of species A, (4) followed by exposure to specific antiserum of species B, (5) followed by testing with antiglobulin serum prepared by injecting species A with serum globulin of species B. Alternatively, serum from a third species (C) may be used for preliminary incubation in step 3 provided the antiglobulin serum used in the last step (5) is first neutralized with serum from this third species (C).

Studies employing a system involving tanned egg albumin (Ea) sensitized R.B.C. preliminary incubation with human serum, exposure to rabbit anti-Ea serum, and testing with hen antirabbit globulin serum neutralized with normal human serum have yielded specific reactions in substantial titers (e.g., 1:3,200). Preparations of serum albumin or gamma globulin cannot be substituted for whole serum or plasma in the preliminary incubation step (3). There is considerable variability in the ease with which the nonspecific adsorbtion globulin from different rabbit sera can be inhibited by human serum. In instances where the amount of the latter required to produce complete inhibition becomes too large to be practical, a differential agglutination titer can be obtained by comparing results with and without preliminary neutralization of the specific antiserum by direct addition of antigen.

The reproducibility of the results is good, though the test is distinctly less sensitive than the direct tanned cell technique (Boyden). It has been developed to study human allergic antibodies which fail to give direct agglutination of tanned erythrocytes sensitized with allergens.

75. THE EFFECTS OF SEROTONIN (5-HYDROXYTRYPTAMINE) UPON SYSTEMIC, PULMONARY, AND CORONARY HEMODYNAMICS AND METABOLISM

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Eighteen mongrel dogs were anesthetized with morphine sulfate (3 mg./Kg. I.M.) followed in one hour by pentobarbital (12 mg./Kg. I.V.). Control cardiac

output (Fick) and coronary blood flow (N_2O application of Fick) were determined; 20 minutes was allowed to elapse before serotonin creatinine sulfate was administered by infusion through a catheter in the right atrium (20 μg/Kg./min.). Five minutes after starting the serotonin administration, cardiac output and coronary flow measurements were repeated, during which time the infusion was continued.

Heart rate increased from 94 to 115/min., M.A.B.P. decreased from 104 to 85 mm. Hg, and mean pulmonary artery pressure changed by +30 per cent. Since cardiac output and stroke volume were unchanged, left ventricular work and total peripheral resistance decreased due to the change in M.A.B.P. Total pulmonary resistance and right ventricular work increased as a function of elevation of pulmonary artery pressure. An increase in ventilation was associated with a significant reduction in carbon dioxide content of arterial, mixed venous, and coronary sinus blood. Coronary flow increased from 71 to 85 ml./100 Gm./min. Coronary flow/beat ratio was unchanged. Coronary vascular resistance decreased significantly. Coronary sinus oxygen content increased from 3.1 to 5.9 vol. per cent. Thus the decrease in myocardial oxygen extraction associated with the increase in coronary flow resulted in myocardial oxygen consumption remaining unchanged. Myocardial carbon dioxide excretion was maintained in a similar manner. The index of efficiency (left ventricular work/$CMRO_2$) decreased. Coronary blood flow/left ventricular work ratio increased by 65 per cent.

Under the experimental conditions described, serotonin acts as a peripheral and coronary vasodilator and has a vasoconstrictor effect upon the pulmonary vascular system. The decrease in coronary vascular resistance was associated with a reduction in M.A.B.P., increase in coronary flow, with myocardial oxygen consumption remaining unchanged. This is the pattern of a "benign" coronary vasodilator.

76. MAGNESIUM DEFICIENCY IN CHRONIC ALCOHOLISM

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Symptomatic magnesium deficiency states have been reported in experimental animals, in livestock, and in man. Since magnesium is an ubiquitous element, spontaneous deficiency in man is unknown. Deficiency in man usually occurs after prolonged parenteral fluid treatment without magnesium replacement and in chronic alcoholism. Low serum magnesium levels have often accompanied magnesium deficiency states, but not invariably. Since serum magnesium levels are static measurements and are unpredictably normal even when deficiency exists balance studies were undertaken in alcoholics to obtain evidence of total body depletion of magnesium.

In control patients with peptic ulcer on a diet of milk, or of milk and cream, toast, and eggs, magnesium balances were all negative with minus values of 9, 3, 26, and 19 mEq. obtained during 6-day study periods. The negative balances averaged 12 per cent of intake. When 2 control patients were given intramuscular magnesium sulfate, it was promptly excreted in the urine with no significant retention, only 10 and 4.8 mEq. of magnesium being retained (4.5 and 3.1 per cent of intakes, respectively).

In 2 alcoholic patients studied for 8 days, with diet and sedation the only therapy, positive balances of 39 and 66 mEq. were measured, averaging 39 per cent of intake. Body magnesium conservation was frequently reflected in strikingly low urinary magnesium excretion. Five alcoholic patients were treated with diet and intramuscular magnesium sulfate. Positive balances of 170, 55, 35, 113, and 74 mEq. in a period of 6 days were obtained, using magnesium sulfate in amounts ranging from 48 to 222 mEq. The positive balance averaged 37.2 per cent of intake (range 22.5 to 51.3 per cent).

These results are further evidence of total body magnesium depletion in chronic alcoholism and lend support for the rational use of magnesium salts as part of the treatment of acute and chronic alcoholic states.

77. THE EFFECT OF LONG-TERM ADMINISTRATION OF AN ANABOLIC DRUG, NORETHANDROLONE, ON THE BLOOD LIPIDS OF CHRONICALLY UNDERWEIGHT INDIVIDUALS

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Serial studies of blood lipids were carried out before and during continuous long-term administration of norethandrolone (Nilevar), to 57 chronically underweight, but essentially healthy, individuals. Statistically significant weight gains in these individuals have been previously reported from this laboratory.

The 57 subjects included: (1) 24 elderly males 55 to 70 years of age and (2) 11 women and 22 men, all between 18 and 40 years of age.

A daily oral tablet containing either 25 or 50 mg. of norethandrolone or a placebo was administered by the double blind technique for periods of 3 to 6 months. Blood samples were drawn for serum cholesterol, lipid phosphorus, alpha and beta lipoprotein, and protein fraction determinations prior to therapy and at monthly intervals during therapy. Serum proteins and lipoproteins were separated by paper electrophoresis. The sera for these determinations were frozen and stored until all samples had been obtained. This permitted simultaneous separation and staining of all sera from each subject in order to ensure valid comparisons.

On the 50 mg. norethandrolone dosage, the same changes were observed in both age groups. There was a distinct reduction in alpha lipoprotein but no change in beta lipoprotein levels, with a resultant decrease in the alpha/beta ratio. Both cholesterol and phospholipid levels decreased, but the cholesterol/phospholipid (C/P) ratio increased slightly. Although women initially showed higher alpha lipoprotein and lower cholesterol levels, the changes were similar both in direction and degree to those observed in men.

On the 25 mg. norethandrolone dosage, moderate reductions in both cholesterol and phospholipid levels, together with an increase in the C/P ratio, were observed in both age groups. In the younger subjects, no changes in lipoprotein values were seen. In the older individuals there were proportional decreases in both the alpha and beta lipoproteins with no change in the alpha/beta ratio.

Serum protein fractions remained relatively constant throughout.

These findings suggest that a daily 50 mg. dose of norethandrolone results in blood lipid changes similar to those observed with methyltestosterone. These lipid effects were infrequently seen at the 25 mg. dose level.

78. EFFECTIVENESS OF MONOVALENT INFLUENZA A-PRIME VACCINE DURING 1957 A-PRIME EPIDEMIC

GORDON MEIKLEJOHN, M.D., DENVER, COLO.

Monovalent aqueous influenza A-prime (AA56) vaccine was given to 1,188 and monovalent aqueous influenza B (GL54) vaccine to 1,216 unseasoned military personnel in mid-December, 1956. Antibody response was evaluated by hemagglutination-inhibition tests with strains AA56 and De 1-57. The vaccine was shown to be highly antigenic in tests with the homologous strain (AA56) but produced only a poor response to the heterologous strain (De 1-57) responsible for the epidemic, which was an A-prime virus of Nederland 1956 type. A sharp epidemic of influenza began two weeks after vaccination. Follow-up observations were limited to men who were hospitalized. Diagnosis was based on the serologic tests for influenza A, influenza B, and adenovirus. During the period of the epidemic 46 men in the vaccinated group and 98 men in the control (influenza B vaccinated) group were hospitalized. The number of cases of influenza was 11 (9.2/1,000) in the vaccinated and 62 (50.9/1,000) in the control group. The number of men admitted with other respiratory diseases was 35 (29.5/1,000) in the vaccinated group and 36 (29.6/1,000) in the control group. The protection ratio was 5.5 to 1. Subsequent to the influenza epidemic a considerable number of cases of adenovirous and streptococcal infections occurred in the study group. These were equally distributed between the vaccinated and control groups. In a concurrent study in seasoned troops the incidence of influenza was considerably lower in the control group. A comparable protective effect was not observed. Possible explanations for the difference in results are discussed.

79. INVESTIGATIONS ON THE METABOLISM OF HYDROCORTISONE IN PATIENTS WITH SHOCK DUE TO INFECTION

JAMES C. MELBY, M.D. (BY INVITATION), RICHARD H. EGDAHL, M.D., PH.D. (BY INVITATION), AND WESLEY W. SPINK, M.D., MINNEAPOLIS, MINN.

Adrenal function has been studied in patients with shock due to infection by measuring the plasma concentrations of endogenous hydrocortisone and by measuring the rate of removal of injected hydrocortisone from the plasma. Hydrocortisone is the principal adrenal cortical secretory product in man and was measured by the method of Silber and Porter. Patients in shock displayed very high levels of hydrocortisone. The normal controls had concentrations averaging 14 μg per cent, whereas in 20 patients with shock the average level was 63 μg per cent. Although the patients in shock had high concentrations of 17-hydrocortisone, further stimulation of the adrenals with ACTH resulted in levels of over 100 μg per cent. These observations in man were confirmed in a group of 13 dogs by direct measurement of hydrocortisone in adrenal vein blood after shock had been produced by endotoxin from gram-negative bacteria. These studies in man and in the dog show that shock is not associated with adrenocortical exhaustion.

The rate of clearance from the plasma of infused hydrocortisone varies directly with the rate of its catabolism and is expressed in terms of its biologic half-life in the plasma. The mean plasma half-life of infused hydrocortisone of normal humans differed markedly from that found in patients with shock.

Furthermore, there was considerable difference in the values in those patients with shock who recovered, and those who expired. The mean plasma half-life of hydrocortisone in the normal subject was 120 minutes; in 8 patients with shock, and who eventually recovered, the half-life was 85 minutes; and in 12 patients who died, the half-life was greater than 300 minutes. In interpreting the significance of these findings it is suggested that the half-life of hydrocortisone is prolonged in the moribund patient because of impaired hepatic function that occurs in the agonal state, while the decreased half-life in those individuals who recovered may represent an accelerated utilization of hydrocortisone in response to an increased demand of the tissues.

80. THE ABSORPTION, DISTRIBUTION, AND EXCRETION OF MECAMYLAMINE IN RATS AND MAN

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Mecamylamine is a secondary amine used in the treatment of hypertension. Observations were made on its absorption, distribution, and excretion in man and animals.

Balance studies in patients with hypertension indicate that mecamylamine was completely absorbed from the gastrointestinal tract. Its distribution in the body was studied by injecting it intraperitoneally into rats. Analysis of various organs was made before and 6, 12, 18, and 24 minutes after injection. The highest concentrations were noted in the spleen; decreasing concentrations followed in the liver, lungs, kidney, heart, and skeletal muscle. Mecamylamine rapidly appeared within cells and in combination with nucleoproteins. This distribution is different from that of hypotensive agents, such as hexamethonium and pentolinium, which concentrate in the extracellular fluid. The intracellular concentration of mecamylamine is similar to what is seen with the antimalarial drugs, such as mepacrine and chloroquine.

Eight hospitalized patients with severe hypertension were given steady maintenance doses (12.5 mg. to 37.5 mg./day) of mecamylamine. Serial observations were made simultaneously of blood pressure, urinary pH, and concentration of mecamylamine in the serum and urine. Studies were made during a period of acid urine excretion and later during a period of alkaline urine excretion. When the urine was strongly acid, large quantities of mecamylamine were found in the urine. This was accompanied by a rise in blood pressure. When the urine was very alkaline, only small quantities of mecamylamine were excreted, and the blood pressure was reduced.

To study this phenomena further, 34 rats were injected intraperitoneally (25 mg./Kg. body weight) twice daily for 3 days. Sixteen were given ammonium chloride to produce an acid urine. The remaining 18 were given Diamox or sodium bicarbonate to alkalinize the urine. In the group excreting an alkaline urine, there was marked retention of mecamylamine, and 13 rats died in apparent hypotensive crisis. In the group which excreted an acid urine, only 2 deaths occurred. This difference is highly significant ($P < 0.001$).

The relationship of pH differences to the transfer mechanism of mecamylamine across the cell membrane will be discussed.

81. URINARY FINDINGS IN COLLEGE ATHLETES FOLLOWING CONTACT AND NONCONTACT SPORTS

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These preliminary observations record urinary findings in college athletes before, immediately after, and 7 to 9 days following participation in three major fields of intercollegiate athletics.

Football.—Prior to the game, 17 players showed normal routine urinalyses. Immediately after, all 17 showed albuminuria, ranging from a trace to 3+. Three demonstrated microscopic hematuria. Nine showed granular or hyaline casts, or both. Sixteen players returned for examination 9 days after the contest. One player still had 1+ albuminuria and one had 4+ albuminuria. Two players still showed microscopic hematuria. No casts were demonstrated in the last examination.

Track (cross-country).—Four runners had negative urinalyses before the contest. Immediately following, 3 had 3+ albuminuria, and one had a 1+ reaction. Three demonstrated microscopic hematuria. One had granular casts and one had hyaline casts. Two reported for study 9 days later, and their urine samples were normal.

Basketball.—Five players had negative urinalyses before the game. Immediately after, albuminuria was demonstrated for 4, ranging from a trace to 3+. There was no hematuria. Three specimens contained granular casts and one had hyaline casts. Seven days after the contest, all 5 were normal.

As has been previously reported, it is apparent that albuminuria and cylindruria are common findings following athletic contests and usually subside in a short time after the resumption of average daily activities. It is noteworthy that bodily contact is not necessary for the production of albuminuria and cylindruria.

82. SEQUESTRATION OF TRANSFERRIN DURING IRON ABSORPTION IN IRON DEFICIENCY

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Using the Barkan and Walker method for serum iron (SeFe) and the Rath and Finch method for unsaturated iron binding capacity (UIBC), the total iron binding capacity (TIBC) was reported as lowered in 14 iron-deficient subjects during the absorption of oral ferrous sulfate (J. LAB. & CLIN. MED. **48**: 926, 1956). Exception was taken to these results based on the possibility of poor recovery of iron by the Barkan-Walker method at high concentrations of SeFe. The potential import of the observations led to a repeat of the study on 16 iron-deficient patients using 3 methods simultaneously for SeFe (Barkan-Walker, Moore, and Kitzes) and two methods for UIBC (Rath-Finch and Feinstein).

The Barkan-Walker and Moore methods were compared at low, normal, and high SeFe by analysis of variance. Using all values a significant difference was noted between the two methods (p 0.005). This difference was due to marked

differences in the samples from two subjects. Deleting those values (Chauvenet's principle) no difference was found between the two methods (p 0.40). No difference was found between the Rath-Finch and Feinstein methods for UIBC (p 0.1). The standard error of the Rath-Finch method on duplicate determinations was 5.9 μg/100 ml.

After 1.3 Gm. of ferrous sulfate by mouth (gelatin capsule, fasting state) a significant lowering of TIBC occurred in 14 of the 16 patients. Similar results were obtained with the Rath-Finch method plus the three SeFe methods. The mean maximal change of TIBC at 2 to 4 hours was: Barkan-Walker –101 ± 52; Moore –87 ± 55; Kitzes –61 ± 29. Concomitantly the SeFe rose. Simultaneous use of Feinstein and Rath-Finch methods in 4 subjects yielded similar lowering of the TIBC in 3. Greatest lowering of transferrin occurred in patients exhibiting tissue changes of iron deficiency.

A transient lowering of transferrin during iron absorption supports the hypothesis considering transferrin in two phases in equilibrium, a circulating and a sequestered phase. In iron deficiency, according to this concept, the equilibrium is shifted toward the circulating phase producing a high TIBC and low SeFe. In hemochromatosis the sequestered phase may be favored, thus a tendency toward a lowered TIBC while the SeFe is elevated.

83. DEMONSTRATION OF AEROBIC OXIDATIVE GLUCOSE METABOLISM IN THE ERYTHROCYTE

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(INTRODUCED BY R. WEISMAN, JR., M.D.)

This study indicates that mature human erythrocytes utilize glucose aerobically by the phosphogluconic pathway as well as anaerobically by the Embden-Meyerhof pathway. Although previous investigations have indicated that enzymes necessary for the phosphogluconic oxidation pathway are present in the erythrocyte, attempts to demonstrate aerobic glucose utilization have hitherto been unsuccessful.

Normal human erythrocytes, washed free of leukocytes, suspended in autologous serum, and incubated with C^{14} glucose produce $C^{14}O_2$, indicating that oxidative glucose utilization via the phosphogluconic pathway occurs. Incubation with glucose-1-C^{14} results in one hundred times the radioactivity in the $C^{14}O_2$ as did glucose-6-C^{14} indicating selective decarboxylation of the one carbon position of the original glucose molecule. $C^{14}O_2$ would not be formed from glucose-1-C^{14} if the glucose metabolism were entirely via the Embden-Meyerhof pathway.

Determination of glucose metabolized by the phosphogluconic oxidation pathway is based on the quantity of glucose utilized and the quantity of $C^{14}O_2$ recovered when blood is incubated with glucose-1-C^{14}. Ten to twenty per cent of the glucose metabolized at pH 7.5 occurs via the oxidative pathway, and this is increased to 20 to 50 per cent at pH 7.1. The relative increase occurs because the amount of glucose metabolized by the anerobic Embden-Meyerhof pathway is significantly reduced at the lower pH, while the contribution of the aerobic oxidation pathway is relatively unchanged. This marked effect of pH may explain in part the failure of earlier investigators to demonstrate significant aerobic oxidative metabolism of glucose in erythrocytes. It has also been found that the oxygen saturation of the blood has a direct effect on the aerobic phosphogluconic pathway. A decrease in the oxygen saturation results in a decrease in the amount of glucose metabolized by the aerobic oxidative pathway.

These studies indicate that there is significant aerobic oxidative glucose metabolism in mature human erythrocytes. The demonstration of the functional existence of aerobic oxidative metabolism may explain the utilization by erythrocytes of ribose-5-phosphate of nucleosides as a source of carbohydrate.

84. SOME CORRELATES OF BLOOD PRESSURE LABILITY IN ESSENTIAL HYPERTENSION

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(INTRODUCED BY MARK H. LEPPER, M.D.)

Acute and seemingly spontaneous variations in blood pressure may mask the true course of hypertensive disease or obscure drug effects. Knowledge of the nature and mechanism of such changes is essential to understanding the natural history and pathologic physiology of hypertension. This study explored some psychologic and physiologic correlates of blood pressure lability in 20 Negro women with essential hypertension.

Initially, it was postulated that lability might be related to severity of the disease or correlated with unusual sensitivity to changes in concentration of endogenous pressor agents. Labile hypertensives might exhibit greater "neurogenic" control of blood pressure, or a greater vulnerability to psychologic or painful stimuli. There might be a predictable association between blood pressure lability and certain personality traits.

The standard deviation of 5 random blood pressure determinations was chosen as the index of lability, and the subjects ranked accordingly. The above hypotheses were then tested by ascertaining blood pressure responses to the following procedures.

1. Intravenous infusion of 10 γ/min. levarterenol
2. Intravenous infusion of 5 units/min. angiotonin
3. Intravenous injection of 400 mg. tetraethylammonium chloride
4. Cold pressor test
5. A standard psychologically stressful interview

Some dimensions of personality and behavior were assessed by means of interviews and psychologic tests including the Minnesota Multiphasic Personality Inventory.

There was no significant correlation between lability and the following: severity of the disease; pressor responses to levarterenol, angiotonin, or cold.

The more labile hypertensives exhibited a significantly greater fall in systolic blood pressure after tetraethylammonium chloride. There was a striking positive correlation between pressor responses to the psychologically stressful interviews and the lability rankings. Blood pressure variability also correlated significantly and positively with scores on the hysteria, hypochondriasis, and psychasthenia scales of the Minnesota Multiphasic Personality Inventory. Behavior data and interviews indicated a greater day-to-day variability in feeling state and behavior among the more labile hypertensives.

In summary, the subjects whose blood pressure was more labile were distinguished by unusual vulnerability to psychologic stress, a specific psychologic test score pattern, wide fluctuations in affect, and a probable greater degree of "neurogenic" control of blood pressure.

85. ELECTROLYTE METABOLISM IN EARLY ESSENTIAL ARTERIAL HYPERTENSION

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Electrolyte metabolism in essential arterial hypertension is of interest because alterations in cellular electrolytes could be responsible for the increased vascular tone found. Studies of exchangeable electrolytes in hypertensive patients have been fragmentary or have included patients with heart or renal failure so that a clear picture of electrolyte distribution in the early stage has not been demonstrated.

Using Na^{24}, K^{42}, and Br^{82}, we studied exchangeable sodium (E_{Na}), potassium (E_K), chloride (E_{Cl}), and the 20-minute distribution of Na^{24} after intravenous administration for an estimate of extracellular space (ECF) in 12 men with early, uncomplicated essential hypertension and in 18 men of similar age and height without cardiovascular or renal disease who served as controls. To better classify the groups we measured glomerular filtration rate (C_{In}), renal plasma flow (C_{PAH}), clearance of electrolytes, and the response to an intravenous salt load (1 ml. 5 per cent NaCl per 0.01 M^2 B.S.A.). The hypertensive group had a mean increase of blood pressure of 27 mm. Hg systolic and 24 mm. Hg diastolic, was 28 pounds heavier ($p < 0.01$), and had > 45 per cent more adipose tissue ($p < 0.01$) than the control group. ECF space was smaller ($p < 0.05$) as was E_{Na} ($p < 0.01$) and E_{Cl} ($p < 0.05$) in the hypertensives when calculated as mEq./Kg. of body weight. There was a suggestive decrease in E_{Na}/E_K ratio, and a similar increase in excretion of sodium after loading in the hypertensive patients ($p > 0.1$). C_{PAH} was decreased (667 versus 542 ml./min.) in the hypertensives ($p < 0.05$), but there was little change in C_{In} between the two groups.

The changes in ECF, E_{Na}, and E_{Cl} probably are secondary to the obesity of the hypertensive group. The relative increase in E_K and, therefore, the decrease in E_{Na}/E_K ratio cannot be explained by the fatness of the hypertensive group. These data are consistent with the theory that there is little total change in body electrolytes in early essential hypertension, although there may be a change in their relative concentration in compartments of the body. The study demonstrates that many of the reported abnormalities of electrolyte metabolism are absent or blunted in mild, early hypertensives.

86. EFFECTS OF HEPARIN AND DICUMAROL ON ATHEROGENESIS IN CHOLESTEROL-FED COCKERELS

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(WITH THE ASSISTANCE OF D. FRIEDMAN AND P. JOHNSON)

In view of the growing utilization of heparin and Dicumarol in the long-term treatment of human atherosclerotic disease, the effects of these drugs were assessed on experimental atherogenesis in cholesterol-fed cockerels.

Three series of experiments were accomplished using depot heparin (2, 10, and 20 mg./bird/day) in chicks ingesting mash supplemented with 0.5, 1.0, and 2.0 per cent cholesterol plus 5 per cent cottonseed oil for 5 weeks. Heparin did not suppress diet-induced hypercholesterolemia, nor did it lower the level of any beta lipoprotein class in intact, alloxanized, or depancreatized cockerels. Coronary and aorta atherogenesis were also unaffected.

In two series of experiments with oral Dicumarol (4 to 30 mg./bird/day) for 5 weeks, aggravation of cholesterol-induced coronary atherosclerosis occurred; no effects were observed on hyperlipemia or aorta atherogenesis. In one experiment, heparin and Dicumarol counteracted the ability of estrogens to prevent coronary lesions, i.e., both anticoagulants apparently exerted a positive atherogenic effect.

Despite prolongation of clotting time, no evidence was obtained of hemorrhage into plaques.

These findings suggest that heparin and Dicumarol, in the presence of the prerequisite dietary and lipid metabolic alteration, may potentiate atherogenesis in the chick.

87. HEMOGLOBIN M DISEASE

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Hereditable hemoglobinopathies among Caucasians of Nordic extraction are very rare. A globin abnormality leading to faulty oxygenation of hemoglobin was found by Horlein and Weber (Deutsche med. Wchnschr. **73**: 476, 1948) in a German family that had exhibited cyanosis for four generations. This hemoglobin, designated by Singer (Am. J. Med. **18**: 633, 1955) as Type M was described by Gerald, Cook, and Diamond (Science **126**: 300, 1957) in a cyanotic family of German extraction and characterized as an abnormal methemoglobin.

This report is the study of a Swiss family with the hemoglobin M defect. The index case, a 33-year-old woman, cyanotic since birth was shown to have splenomegaly and the hematologic manifestations of mild hemolytic anemia. The identical cyanotic defect was present in the patient's father and son. A daughter was normal.

The blood was grossly chocolate brown, but methemoglobin could not be demonstrated by the usual spectrophotometric or chemical means. The presence of methemoglobin reductase was demonstrated by ability of the patient's intact erythrocytes to reduce nitrite induced methemoglobin to hemoglobin in vitro.

Paper electrophoresis of hemoglobin showed two clear-cut components. The faster migrated in the position of adult (A) hemoglobin and was chocolate brown in color, implying faulty oxygenation. The slower fraction moved with the velocity of C hemoglobin and was bright red in color. Eleven per cent of hemoglobin was alkali resistant (Type F).

It seems likely that this defect represents a type of dominant hereditary hemoglobinopathy (Type M) characterized by mild hemolytic anemia and cyanosis. The properties of Type M hemoglobin include slow electrophoretic migration and inability to transport oxygen.

88. THE EARLY PATHOGENESIS OF EXPERIMENTAL HISTOPLASMOSIS

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The natural route of infection with the organism *Histoplasma capsulatum* is generally considered to be via the respiratory tract. The chlamydospore of

the mycelial phase is inhaled, resulting in a primary pulmonary infection. Dissemination of the organisms throughout the reticuloendothelial system occurs following conversion of the spore to the invasive yeast phase.

This study was planned to demonstrate the initial histologic reaction to the histoplasma spore within the mouse lung during which the conversion of the spore to the yeast phase takes place. The natural mode of infection was simulated by introducing a saline suspension of mature tuberculate chlamydospores into the nares of ether-anesthetized white female mice. Approximately 700 fifteen gram mice were each infected with 5,000 to 20,000 spores. Plating techniques proved 60 to 80 per cent viability of the spores. Groups of 3 to 6 mice were sacrificed at frequent (3 hour) intervals over a 10-day period for pathologic and cultural study. Others were observed for a more extended period (56 weeks) and sacrificed at 2-day intervals. Lungs were fixed by the intratracheal injection of Zenkers-formol solution. Hematoxylin-eosin, hematoxylin-eosin-azure, Hotchkiss-McManus, and Gridley staining techniques were employed. Organ cultures on routine media showed that by 3 days dissemination had occurred.

Microscopic lung sections observed in sequential pattern beautifully illustrated the early tissue reaction to the spore and its disintegration. Mature typical tuberculate chlamydospores approximately 10 μ in size were seen in many alveoli. Within 6 hours they were "rimmed" by polymorphonuclear leukocytes. By 18 hours alveoli containing spores had become clogged with polymorphonuclear leukocytes and epithelioid cells. Adjacent alveoli contained a few free macrophages. Exudate had spread into contiguous alveoli by 36 hours. At 48 hours spore tubercles protruded prominently and an internal trabeculation and segmentation occurred. By 72 hours some spores had ruptured and portions of the chitenous wall were phagocytosed by macrophages. At this time encapsulated yeast cells also were seen in free macrophages. Only a few spores persisted beyond 5 days and showed variable modes of disintegration. The widespread distribution of the progressive granulomatous infiltrates in proximity to the bronchioles was notable. Foci of infection in other organs indicated by small granulomas could be observed by 6 days.

89. FOOD ADDICTION AND ALCOHOLISM

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Most of us adapt to our diet without becoming allergic. Some become sensitized to specific foods, manifesting a pattern of symptoms largely dependent upon *how frequently* and *how much* is eaten per dose.

In food addiction (high degree of chronic marked food sensitivity), one adapts to oft-repeated, regularly spaced, relatively constant-sized to gradually increasing feedings of a given food by being "picked up" immediately but "hung over" several hours later. Progressively more frequent specific doses are required to relieve "hang-overs" and perpetuate "pickups"—the worse the "hang-over," the more satisfying the "pickup."

Avoidance breaks this adaptation, often resulting in temporarily troublesome withdrawal effects. After these subside, an *isolated* dose precipitates an immediate reaction, the basis of the individual food ingestion test. This explains why allergy to such *rarely eaten foods* as shrimp is readily recognized, whereas sensitivity to milk or *common regularly eaten foods* is not suspect.

One customarily picked up from the usual amount of a given food often develops an acute reaction from a large or more rapidly absorbed dose. For instance, a spaghetti dinner or a drink of beer often upsets the bread-eating wheat-barley sensitive individual adapted to lower dosage. But with chronic exposures, such as daily beer drinking, these immediate postexposure upsets

taper off as one becomes adapted to and dependent upon progressively larger doses. With increasing sensitivity, adaptation to a given food or food containing beverage levels off and finally fails. Size of dosage is then decreased to avoid immediate reactions. For example, a corn-sensitive alcoholic may cease: eating popcorn while drinking; simultaneous eating and drinking; using beer for bourbon; bourbon for Scotch; Scotch for gin; gin for wine, before finally abstaining.

Specifically diagnosed alcoholics are usually allergic to corn, wheat, potato, grape, cane, beet, or yeast, but any food such as coffee may be involved. The abstaining alcoholic's hang-overs and cravings, continued by specific foods, may be lessened or abolished by avoiding both potable and edible sources. Although social drinking of compatible beverages by alcoholics has been tried successfully, errors in specific avoidance or other allergic exposures may precipitate drinking binges ending in pathologic intoxication.

90. THE INFLUENCE OF THE PULMONARY CAPILLARY BLOOD VOLUME, GAS TENSIONS, AND HEMATOCRIT ON DIFFUSING CAPACITY FOR CARBON MONOXIDE

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(INTRODUCED BY OVID O. MEYER, M.D.)

Recently it has been shown that the diffusing capacity of the lung for CO depends not only on the physical properties of the alveolar capillary membrane but to a comparable extent depends on pulmonary capillary blood volume and on the rate at which erythrocytes within the pulmonary capillary bed can take up CO. If the diffusing capacity of the lung for CO (D_L) and the in vitro rate of combination of CO with intracorpuscular hemoglobin (θ) are known for several different oxygen tensions, it is possible to assess the relative importance of these factors, and to determine the true diffusing capacity of the pulmonary membrane (D_M) and the pulmonary capillary blood volume (V_C). Studies were performed on 34 normal subjects and patients. An increase in alveolar oxygen tension decreased D_L without change in the physical property of the membrane since O_2 completes with CO for hemoglobin. Hypercapnia increased D_L largely as a result of an increase in V_C. In patients with anemia and no evidence of pulmonary disease, D_L decreased as a result of a reduction in the pulmonary hematocrit and in some instances a marked reduction in D_M. In patients with diffuse interstitial pulmonary fibrosis or pulmonary emphysema D_L decreased, owing, in the main, to a decrease in D_M although in some instances there was also a reduction in V_C. In patients with pulmonary hypertension there was a proportionate reduction in D_M and V_C. In patients with pulmonary congestion as a result of heart disease the average D_L was normal, in spite of a reduction in D_M owing to a considerable increase in V_C. These results indicate that the pulmonary capillary blood volume, gas tension, and hematocrit have a significant effect on pulmonary diffusing capacity for CO.

91. THE ROLE OF HAGEMAN FACTOR IN THE INITIATION OF CLOTTING BY GLASS

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The forces which maintain the fluidity of blood within blood vessels are unknown. Normal human cell-free plasma clots slowly in silicone-coated tubes

but clots rapidly in glass. Margolis and Shafrir and deVries showed that the effect of glass was mediated at least partially by a component of plasma which was stable at 56° C. and was not adsorbed by barium sulfate or albumin hydroxide. Among described clotting factors, only plasma thromboplastin antecedent and Hageman factor have these characteristics. In agreement with these earlier studies, experiments demonstrated that the plasma of patients with a variety of coagulative disorders reacted with glass. These abnormal plasmas, treated with glass, accelerated the clotting of normal plasma. Only the plasma of patients with Hageman trait was deficient in the glass-reactive property. It has been shown previously that plasma of patients with Hageman trait behaved as though devoid of Hageman factor, which is required in vitro for the optimal development of plasma thromboplastic activity. These studies suggested that acceleration of clotting by glass required the presence of Hageman factor.

The mechanism by which glass "activated" Hageman factor was studied by taking advantage of the observation that duck plasma seemed deficient in Hageman factor and its clotting was not enhanced by glass. When duck and human plasma were mixed before exposure of glass, the clot-promoting effect of glass was inhibited. However, duck plasma did not inhibit the acceleration of clotting by human plasma which had previously been treated with glass. The same results were obtained using plasma from patients with Hageman trait instead of duck plasma. Thus plasma from ducks or from patients with Hageman trait appeared to inhibit the "activation" of Hageman factor by glass.

These observations suggest the following tentative hypothesis. The fluidity of blood within blood vessels is maintained by one or more inhibitors directed against Hageman factor. Exposure of blood to glass removes this inhibition. The uninhibited or "activated" Hageman factor then initiates clotting. Since patients with Hageman trait do not have a bleeding tendency, other mechanisms may initiate clotting when blood leaves the vessels as the result of injury.

92. RATES OF TRANSFER OF Rb^{86}, K^{42}, K^{39}, Cl^{36}, Cl^{35}, Na^{22}, AND Na^{23} ACROSS A BLISTER SURFACE ON THE FOREARM OF NORMAL MAN AND PATIENTS WITH CHRONIC CONGESTIVE HEART FAILURE

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The base of a cantharides blister on both forearms of control subjects and patients with congestive heart failure was used to study the kinetics of electrolyte exchange across a living surface in intact man. Although multicellular and complex, this "membrane" is uniform, reproducible, and readily adaptable to such studies. The effects of varying the constituents of the fluid in the extracorporeal blister compartment (e.b.c.) upon the rates of transfer of Rb^{86}, K^{42}, K^{39}, Cl^{36}, Cl^{35}, Na^{22}, and Na^{23} were observed.

With Rb^{86} in the subject's sera in the e.b.c., concentration half-time of Rb^{86} was 91.3 ± 24.5 and 91.3 ± 16.6 minutes for left and right forearms, respectively. Rates of exchange for control subjects and patients with failure were the same. Since Rb^{86} and K^{42} studies simultaneously had the same rates of transfer, the rate of exchange was calculated for K^{39} from the Rb^{86} tracer studies at 0.00031 mEq. K per 10 cm.^{2}/min.

With 5 per cent glucose, Rb^{86} and K^{39} (4 to 50 mEq./L.) in the e.b.c., the rates of exchange were: K^{39}, 0.0027 ± 0.0009; Cl^{35}, 0.0075 ± 0.0014; and Na^{23}, 0.0078 ± 0.0017 mEq./10 cm.^{2}/min.

Simultaneous rates of transfer of Rb^{86} into and out of e.b.c. were equal with both changing and constant arterial concentrations of Rb^{86}. The lymphatic

vessels contributed negligibly to removal of isotopes from e.b.c. Na^{22} and Cl^{36} transferred from e.b.c. at same rates as Na^{23} and Cl^{35} entered from the blood. Differences in rates of transfer of Na^{22} and Rb^{86} and of Cl^{36} and Rb^{86} existed when combined in the same test solution.

Results showed the following:

1. Rb^{86} is reliable as tracer of K^{39} in capillary transfer rates.
2. Blister base is reproducible and adaptable as model for such studies in man.
3. Enormous amounts of electrolytes enter and leave the vascular system; about 60,000 pounds of NaCl and 2,000 pounds K daily.
4. Transfer capacity is increased by higher concentrations than in normal serum, 16,000 pounds K daily.
5. Rates of transfer are limited by rate of circulation and mixing.

93. THE QUESTION OF VASCULAR HYPERRESPONSIVENESS IN ARTERIAL HYPERTENSION

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One current theory about hypertension holds that the muscle fibers of arterioles are hyperresponsive to the normal amounts of norepinephrine secreted by the sympathetic nerve endings and to the normal amounts of circulating pressor substances. The evidence supporting this is that much less norepinephrine is required to produce a given decrease in blood flow of the nail-fold, the conjunctiva, or the hand in hypertensive patients than in normotensive patients. Such in vivo experiments cannot distinguish between "real" and "apparent" hyperresponsiveness, however. By "real" hyperresponsiveness we mean an increased per cent shortening of arteriolar smooth muscle after a given dose of pressor agent. By "apparent" hyperresponsiveness we mean a greater than normal change in peripheral resistance or flow accomplished by a normal per cent shortening of arteriolar muscle. We and Folkow have postulated that in arterioles with an increase in wall thickness due to waterlogging or hypertrophy, the same degree of muscle shortening will cause a greatly increased resistance to flow. Moreover, Folkow and Mendlowitz have evidence that such an increase in wall thickness probably occurs in human hypertension. Leaving out the possibility of a thickened arteriolar wall, we have calculated that a decrease in lumen size per se as a result of already shortened circular muscle will cause an exaggerated increase in flow resistance for a given amount of additional muscle shortening. For these reasons it seemed desirable to test hyperresponsiveness more directly. Spirally cut strips of aorta were obtained from both normotensive and hypertensive rats and suspended at 0.5 Gm. tension. Norepinephrine was added and the resulting increased tension was measured isometrically. The aortic strips from 26 normotensive rats developed a mean additional tension of 0.43 Gm. with norepinephrine. The strips from 19 hypertensive rats developed tensions identical with those in the normotensive group. The strips from 10 other hypertensive rats developed between 0.07 and 0.22 Gm. tension. Absolutely no hyperresponsiveness was seen in renal, desoxycorticosterone, or postdesoxycorticosterone hypertension. The muscle of the aorta in hypertensive rats may or may not be typical of all arterial smooth muscle. However, it generally shows normal responsiveness to norepinephrine.

94. PROTEIN METABOLISM IN INFECTION

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(INTRODUCED BY WILLIAM H. DAUGHADAY, M.D.)

The pattern of abnormal protein metabolism in acute infections was studied by use of isotope techniques.

Pneumonia was induced in rats by intrabronchial instillation of Group A beta hemolytic streptococci. Animals serving as controls were intubated without instillation of microorganisms. All animals were fed a normal diet until the time of the operation and were starved thereafter. N-15 glycine was given intraperitoneally either in divided doses for 3 days before the operation or as a single dose after the pneumonia was fully established. Animals were killed at intervals up to 160 hours. The proteins of the liver, kidney, and spleen were measured for the total amount present and N-15 enrichment. In one experiment, N-15 concentration of muscle protein was also measured. Urines were analyzed for nitrogen and N-15 enrichment.

The urinary nitrogen excretion in animals with pneumonia was increased from 22 to 120 per cent. The concentration of N-15 in the urine was lower but the total amount of N-15 in the urine was greater in the animals with pneumonia than in the control animals.

The N-15 concentration in the liver and kidney proteins was lower in the animals with pneumonia, both when the N-15 was given before and after the induction of pneumonia. In one experiment, the concentration of N-15 was also decreased in muscle protein in the animals with pneumonia. The total protein content of the livers of animals with pneumonia remained normal while that of control animals decreased greatly. However, the total amount of N-15 in the liver was the same in both groups and the estimated turnover half-time of liver protein was unaffected by pneumonia.

The data suggest that the predominant effect of infection is the mobilization of amino acids from muscle to the liver. This would make available large quantities of amino acids both for synthesis of protein in the liver and for oxidation to urea, and would explain the increased net synthesis of liver protein acid nonprotein nitrogen excretion observed under the conditions studied. Catabolism of liver protein was unaffected by infection.

95. CHANGES IN BODY COMPOSITION DURING THE COURSE OF ACUTE ANTERIOR POLIOMYELITIS

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This study was designed to determine the changes in body composition which occur during the course of acute anterior poliomyelitis. One to four serial (at 7 to 10 day intervals), simultaneous measurements of total exchangeable potassium (K_e), antipyrine space (TBW) and radiosulfate space (ECF) were made in 20 male and 24 female patients with acute poliomyelitis. From these data were calculated intracellular fluid volume (ICF), lean body mass (LBM), exchangeable potassium per kilogram of lean body mass (K_{LBM}), concentration of potassium per liter of cell water (K_{ICF}), ratio of extracellular fluid and intracellular volumes to antipyrine space ($\frac{ECF}{TBW}$, $\frac{ICF}{TBW}$), and ratio

of extracellular fluid volume to intracellular fluid volume ($\frac{ECF}{ICF}$). Analysis of the clinical courses did not reveal any significant differences between male and female patients with respect to age, severity of illness, or time after onset of the disease the studies were done.

K_e decreased during the course of the illness and this decrease correlated with the clinical estimate of the extent of muscle wasting. Early in the illness, K_{LBM} for males (66.5 ± 10.2 mEq./Kg.) was significantly greater ($p < 0.001$) than K_{LBM} for females (53.4 ± 7.7 mEq./Kg.). During the course of the illness, K_{LBM} decreased significantly for males and females, but by the third serial study there was no significant difference between males (45.3 ± 5.0 mEq./Kg.) and females (46.6 ± 3.1 mEq./Kg.). A significant difference ($p < 0.01$) for K_{ICF} was noted between males (141 ± 29.3 mEq./L.) and females (118 ± 24.7 mEq./L.) at the initial study. During the course of the illness K_{ICF} decreased for males (114 ± 21.2 mEq./L.) and was unchanged for females (119 ± 15.9 mEq./L.); however, the decrease in K_{ICF} for males was not significant. There was a significant increase during the course of the illness in $\frac{ECF}{ICF}$ ratio both for males ($p < 0.005$) and for females ($p < 0.05$); the differences between males and females were not significant.

Significant changes in susceptibility and mortality have been demonstrated in animals inoculated with poliomyelitis virus after administration of gonadal and adrenal hormones. In man, mortality is significantly higher in males. Our observations suggest a mechanism for these experimental and epidemiologic data.

96. NEUROGENIC REGULATION OF THE CIRCULATING PLASMA VOLUME

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A reduction of as much as 25 per cent of the circulating plasma volume (T-1824) and of the blood volume occurs in rabbits, dogs, and chicks subjected to hypothermia (Am. J. Physiol. **167**: 485, 1951). For example, the plasma volume at normal body temperature (41° C.) in the chick is 44 ± 5 ml./Kg. body weight, while after induction of hypothermia (25° C.), the volume falls to 33 ± 5 ml./Kg. These results suggest that hypothermia actuates a mechanism whereby some vascular beds become segregated from the circulating blood stream. The extracellular (thiocyanate) fluid volume is reduced proportionately, perhaps because the extravascular fluids of the tissues supplied by the segregated vascular beds do not participate in the dilution of thiocyanate.

These mechanisms of loculation have been investigated in 137 experiments on chicks subjected to hypothermia. The resulting data suggest that the autonomic nervous system plays a significant role in the volumetric regulation of the circulating plasma. Administration of dibenzyline to normothermic chicks prevents the hypothermic reduction in plasma volume. After hypothermic loculation of some of the plasma volume has occurred, dibenzyline produces a return of the circulating plasma volume to normothermic values. These data indicate that block of the sympathetic effectors results in the addition of a significant quantity of plasma to the circulating blood, with no comparable adjustment of the circulating red blood cell mass. Hydrazinophthalazine and tetraethylammonium have virtually no effect on these measured volumes at either normal body temperature or in hypothermia. Vagotomy is followed by an increased tendency to loculation; atropine has little or no effect. Acute withdrawal of blood is quickly followed by an almost exact restitution of the circulating

plasma volume, indicating an active regulation of the volume of this compartment. The circulating blood cell mass does not return to prehemorrhage levels. The neurogenic regulatory mechanisms elicited by these procedures may also be operative in other disturbances of the circulation.

97. MYASTHENIA AND MALIGNANT MEDIASTINAL TUMORS

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(Introduced by Bayard T. Horton, M.D.)

A group of patients characterized by an unusual type of weakness and a distinctive electromyographic response is described. Most of them either had malignant mediastinal lesions when first examined for weakness or showed evidence of such lesions during the next few months. In some patients, malignant mediastinal tumors have not yet been identified. Small cell bronchogenic carcinoma has been the most frequent histologic diagnosis.

The clinical and electromyographic findings in these patients resemble those found in typical myasthenia gravis but they also differ in several important respects. These differences from the usual case of myasthenia gravis would be of only casual interest except for the fact that the majority of the patients showing these abnormalities have had malignant mediastinal growths. Of particular interest were 4 patients who had normal roentgenograms of the thorax when first seen with this syndrome but whose thoracic roentgenograms became abnormal under observation.

The current series comprises 15 patients. Thirteen had electromyographic findings characteristic of this syndrome, whereas two patients did not have electromyograms but presented the clinical features of the syndrome.

Histologic diagnosis of the thoracic malignant tumor has been made in 7 patients; in 2 patients, a mediastinal mass is apparent roentgenographically but histologic diagnosis has not yet been accomplished. In the remaining 6 patients, no malignant lesions in the thorax or elsewhere have been identified.

Another series of 27 patients with histologically identified malignant mediastinal tumors but without weakness has been examined electromyographically. Abnormalities such as characterize the present group of patients have not been found in any of them.

98. PROPHYLACTIC EFFECT OF IMMUNIZATION WITH ADENOVIRUS VACCINE

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(Introduced by F. M. Davenport, M.D.)

During the winter of 1956-1957 the effect of immunization with adenovirus vaccine on the incidence of acute nonbacterial infections of the respiratory tract was studied in companies of military recruits during their first 8 weeks of basic training. The vaccine was commercially prepared and contained formalin-inactivated adenovirus types 3, 4, and 7; its preparation included passage through a fine-porosity filter. Injection of 1.0 ml. subcutaneously was well tolerated and was followed within a week in approximately 50 per cent of the subjects by a significant and presumably anamnestic rise in complement-fixing antibodies to adenovirus antigen. Between December, 1956, and March, 1957, a total of 6,106 men were immunized and 7,134 in control companies received a dummy injection. Over-all rates of hospital admission for acute respiratory

disease among the control group were twice as large as those among the immunized group, and the incidence of adenovirus infection, as shown serologically, was approximately 5 times as great. The results show that immunization with vaccine of the type used in this study is an effective means of preventing adenovirus infections in military recruits.

99. CHANGES IN IRON METABOLISM IN EARLY CHLORAMPHENICOL TOXICITY

D. Rubin, M.D. (By Invitation), R. E. Botti, M.D. (By Invitation), J. P. Storaasli, M.D., and A. S. Weisberger, M.D., Cleveland, Ohio

Chloramphenicol has been established as a cause of hematopoietic depression. In the absence of ferrokinetic studies such observations have usually been limited to cases of leukopenia or aplastic anemia, the latter frequently being irreversible. Utilizing measurements of Fe^{59} plasma disappearance and incorporation of Fe^{59} into erythrocytes, it has been possible to delineate an early toxic action of chloramphenicol primarily affecting erythrocyte production. Evidence of toxicity may occur in the presence of a normally cellular bone marrow and before any drop in hematocrit takes place.

Studies were performed on 11 patients receiving chloramphenicol. Four patients were found to have abnormalities of Fe^{59} metabolism, consisting of a prolongation in the disappearance of Fe^{59} from the plasma and a delay in the subsequent appearance of Fe^{59} in erythrocytes. Studies performed 10 days or more after discontinuing chloramphenicol demonstrated a reversal of these abnormalities. In 2 patients the hemoglobin and hematocrit determinations were normal at the time that Fe^{59} studies indicated decreased erythropoietic activity. Both patients had a normally cellular marrow, but subsequently exhibited a drop in the hematocrit, which returned to normal after withdrawing chloramphenicol. One patient had a hypocellular marrow and anemia at the time that changes in Fe^{59} metabolism were demonstrated but all findings returned to normal after discontinuing chloramphenicol. In the fourth patient a hypercellular marrow and anemia were present but other factors besides chloramphenicol may be implicated. However, the hematocrit has risen following discontinuance of chloramphenicol.

The 4 patients in whom chloramphenicol was considered to be a hematopoietic depressant exhibited an elevated plasma iron and high saturation of the plasma iron-binding globulin (60 to 90 per cent saturation). These high values reverted toward normal when chloramphenicol was discontinued. Thus determinations of plasma iron and iron-binding globulin saturation may serve as a relatively simple procedure in the detection of early hematopoietic depression due to chloramphenicol.

Since prolonged depression by chloramphenicol may lead to an eventual irreversible marrow aplasia, early detection may be of considerable importance. The techniques employed in this study also may be applicable in screening other drugs for adverse effects on the hematopoietic system.

100. GENERAL HEMODYNAMIC RESPONSES OF MAN TO DRY HEAT EXPOSURE

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The general hemodynamic responses of 16 subjects to a rise in ambient temperature from 73° to 98° F., maintaining the humidity constant at 40 per cent, were studied in a constant temperature room. Eight patients had normal

or enlarged left ventricles with a normal wedge pressure, whereas 8 had enlarged left ventricles and elevation of the wedge pressure. Following the control studies, the room temperature was elevated from, 73° to 98° F. within 22 minutes, and experimental data were collected after 1 and 2 hours of steady heat exposure. Cardiac outputs were determined by the Fick principle. The data were analyzed statistically and a comparison was drawn between the group with normal and the one with elevated wedge pressures.

There was no significant difference in response between the two groups except for the pCO_2, where a significant increase occurred in the patients with elevated wedge pressure. There was a progressive decrease in the pulmonary and brachial arterial pressures, total pulmonary and peripheral arterial resistances, and left ventricular work. Rectal temperature and finger blood flow increased. Minute ventilation, oxygen consumption, arteriovenous oxygen difference and cardiac index showed no significant change. The only evidence of clinical embarrassment occurred in the patients with elevated wedge pressure; in the majority of these, Cheyne-Stokes respiration developed.

The marked decreases in total peripheral and total pulmonary resistances, which ranged on the average from 34 to 39 per cent, occurred in the face of slight but insignificant increases in cardiac output. This would imply a predominantly vasodilating effect by heat in the body as a whole, and a definite vasodilating effect on the pulmonary bed. Studies in progress are designed to assess the changes occurring in the specific vascular territories.

101. STUDIES ON THE ABSORPTION OF ESCHERICHIA COLI ENDOTOXIN FROM THE GASTROINTESTINAL TRACT OF DOGS AS A FACTOR IN THE PATHOGENESIS OF IRREVERSIBLE HEMORRHAGIC SHOCK

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(INTRODUCED BY DONALD W. SELDIN, M.D.)

The persistence of hypotension following retransfusion after prolonged hemorrhage is observed both in man and in experimental animals. Indirect evidence has implicated the absorption of endotoxin derived from gram-negative bacteria within the gastrointestinal tract in the genesis "irreversibility" in dogs. This hypothesis was assessed directly by measuring the absorption of radioactive chromium ($Cr^{51}Cl_3$) labeled *Escherichia coli* endotoxin, prepared according to the Boivin technique, from the gastrointestinal tract of dogs being subjected to "irreversible" hemorrhagic shock. The possibility of increases in the susceptibility of shocked dogs to endotoxin of a magnitude sufficient to mask detection also was evaluated.

The technique of labeling was shown not to inactivate the endotoxin and was sufficiently sensitive to detect the absorption of 1/1,000 or less of the LD_{50} dose of endotoxin in 15 of 19 dogs studied. Absorption of Cr^{51}-labeled endotoxin following administration by gavage or through enterostomies into various segments of the gastrointestinal tract was measured by blood and tissue analyses for radioactivity.

Absorption was not observed following the administration of adequate quantities (2/3 LD_{50} dose) of Cr^{51}-labeled endotoxin either to 13 control dogs or to 19 dogs subjected to external hemorrhage of a degree (40 mm. Hg) and duration (5 hours) accepted as irreversible in at least 80 per cent of dogs. No increase in the susceptibility of 3 dogs to 1/1,000 LD_{50} of endotoxin administered

parenterally after "reversible" hemorrhage (40 mm. Hg for one hour) or of 7 dogs to 1/100 to 1/10 LD_{50} of endotoxin administered parenterally during "irreversible" hemorrhage was demonstrated. While an increase in susceptibility to endotoxin may have occurred in the shocked dogs, the magnitude was not of sufficient degree to mask detection of absorption.

Thus, there is no direct evidence to support the hypothesis that increased absorption of endotoxin from within the gastrointestinal tract is an important factor in the genesis of "irreversibility" following prolonged shock.

102. A HISTOPLASMIN-LATEX AGGLUTINATION TEST

I. Preliminary Results With Animal Sera

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The antibody response to infections with *Histoplasma capsulatum* has been studied most intensively with three types of tests: collodion agglutination, precipitin, and complement fixation. Any of these tests can be performed in a large well-equipped laboratory. However, the smaller hospital and clinical laboratories which receive occasional requests for such studies find these procedures too burdensome either from performance or preparation of antigens.

The purpose of this report is to describe a simple latex agglutination test employing commercially-available Histoplasmin and polystyrene latex particles. It can be performed by the average technician in a few hours. In principle the test is analogous to the collodion agglutination test in that the particles are "coated" with Histoplasmin and then used in a simple agglutination test.

Preliminary studies with sera from infected and normal dogs and monkeys, and sera from immunized and normal rabbits were studied; excellent correlation with the collodion agglutination test was observed. Results with human sera were likewise similar in that the titers were elevated early in the disease and became negative in 2 to 3 months during convalescence. Thus, the test is of most value when the patient has acute disease and when that information is most needed.

The simplicity of the test will be described by appropriate slides.

103. THE EFFECT OF THERAPY ON BLOOD CHOLESTEROL CONCENTRATION IN TUBERCULOUS PATIENTS

J. L. Schmidt, M.D., A. R. Allen, M.D., R. W. Harmon, B.S., D. L. Martin, B.S., and H. D. Brondyk, B.S., North Chicago, Ill., and Selah, Wash.

(Introduced by R. K. Richards, M.D.)

One of us (A. R. Allen) has noted an apparent increase in the incidence of myocardial infarction in tuberculous patients since the addition of isonicotinic acid hydrazide to the armamentarium. In normal male mice another of us (J. L. Schmidt) has noted that the usual effect of administering of isonicotinic acid hydrazide is a small decrease in blood cholesterol concentration.

In ambulatory tuberculous male patients of various races and ranging from 32 to 79 years of age a single admission blood serum cholesterol determination

was made before the start of streptomycin, para-aminosalicylic acid and isonicotinic acid hydrazide therapy. Serial blood serum cholesterol determinations were done at 2- to 4-week intervals for periods ranging from 4 months to a year. One other patient studied only two months showed essentially no change, another studied 13 months showed a decrease except for his last determination. Twelve patients started at various times and studied 4 months or more showed significant increases of blood cholesterol concentration. Peak values showed an increase of over 25 mg./100 ml. in 3 patients, over 50 mg./100 ml. in 7 patients and over 75 mg./100 ml. in 2 patients within 1 to 3 months. Six other male patients, started on the study shortly after therapy already had begun, all showed increases in blood cholesterol of more than 25 mg./cholesterol/100 ml.

The results in a smaller group of women studied to this date have been less consistent. In men, there also appears in many instances a tendency for the blood cholesterol to decline after reaching a peak. In many instances following operative procedures there has been a temporary sharp decline. In most instances the patients have gained weight.

The results of this study indicate that modern drug treatment of tuberculous men is followed by a relatively prompt and statistically significant increase in blood cholesterol concentration. Whether this is due to a generalized stimulation of a positive nutritional state, specific drug effects, or other reasons has not been determined. These results, however, do suggest that further investigation of tuberculous patients may provide useful information regarding the relation of blood cholesterol concentration, drug therapy, and the incidence of atherosclerosis or its complications.

104. DEPRESSED RENAL FUNCTION IN THE CARCINOID SYNDROME: EFFECTS OF SEROTONIN IN NORMAL SUBJECTS AND OF AN ANTISEROTONIN (BROMO-LSD)

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Serotonin inhibits water diuresis in animals and, in large doses, depresses glomerular filtration rate and urine flow. This report describes changes in specific renal functions in two volunteers during intravenous infusions of serotonin given alone or simultaneously with bromo-lysergic acid diethylamide (bromo-LSD), a potent serotonin antagonist in animals. Effects of bromo-LSD were also studied in two patients with the carcinoid syndrome and significant abnormalities of renal function.

Serotonin, 10 to 20 μg/Kg./min., decreased C_{PAH} in two normal subjects; maximum rate of water reabsorption ($T^{c}_{H_2O}$) was unchanged. In 2 of 3 tests, potassium output increased and sodium output decreased. Decreases in $C_{mannitol}$ were observed in two tests with corresponding decreases in urine flow. This sequence accords with that observed in animals given serotonin during osmotic diuresis. In two carcinoid patients, urinary outputs of 5-hydroxyindoleacetic acid averaged 25 mg. and 239 mg. daily. Corresponding rates of C_{PAH} and $C_{mannitol}$ were, respectively, 263 and 65, and 198 and 87 ml./min., indicating severe renal vasoconstriction; $T^{c}_{H_2O}$ was at lower limits of normal. This renal ischemia was unassociated with hypertension, proteinuria, abnormal urinary sediment, or other abnormalities of renal function.

In both normal and carcinoid subjects bromo-LSD further depressed C_{PAH}. Contrary to previous reports, it provoked striking psychic disturbances, resembling that elicited by its congener, lysergic acid diethylamide; these aberrations may have contributed to the renal vasoconstriction.

Impairment of specific renal functions has not been described in the carcinoid syndrome but its occurrence is consistent with observations in animals that small doses of serotonin have variable effects on renal blood flow, while large amounts, such as may circulate in the carcinoid syndrome, cause severe renal vasoconstriction, and even cortical necrosis.

105. A SECOND ENZYME ABNORMALITY IN PRIMAQUINE SENSITIVE ERYTHROCYTES

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Erythrocytes in approximately 10 per cent of adult male and 5 per cent of adult female Negro Americans are deficient in glucose-6-phosphate dehydrogenase. Acute hemolysis results when individuals with enzyme deficient erythrocytes are given primaquine, sulfanilamide, or related drugs. These erythrocytes also have a low total and reduced glutathione content which falls even more when drug is given. Following an acute hemolysis, the glucose-6-phosphate dehydrogenase of erythrocytes does not rise to normal, nor does their glutathione content, yet these cells are resistant to further primaquine induced hemolysis.

Glutathione reductase is present in hemolyzates and keeps most of the glutathione reduced according to the irreversible stoichiometric reaction:

$$*GSSG + *TPNH + H^+ \xrightarrow[\text{Reductase}]{\text{GSSG}} 2\ *GSH + *TPN^+$$

In dialyzed hemolyzates from normal and drug sensitive erythrocytes glutathione reductase activity can be assayed by measuring GSH produced in 14 ml. reaction mixtures containing 6 ml. 0.5 M Tris buffer, 2 ml. 0.05 M Versene,* 10 μM GSSG, 12.6 μM TPNH, and 1 ml. of hemolyzate. Variations in time, temperature, pH, dialysis, and substrate concentration do not affect the results significantly.

In contrast to their deficiency of glucose-6-phosphate dehydrogenase hemolyzates from primaquine sensitive erythrocytes have consistently higher glutathione reductase than do hemolyzates from nonsensitive erythrocytes.

Glucose-6-phosphate dehydrogenase catalyzes the initial step in the hexose-monophosphate shunt, an alternative pathway of carbohydrate metabolism, and also provides a source of TPNH for glutathione reductase.

$$\text{Glucose-6-phosphate} + TPN^+ \xrightarrow[\text{Dehydrogenase}]{\text{G-6-P}} \text{6-phosphogluconate} + TPNH + H^+$$

The increased glutathione reductase may partially compensate for the deficiency of glucose-6-phosphate dehydrogenase by regenerating the available

*GSSG, oxidized glutathione; GSH, reduced glutathione; TPN, triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; Versene, ethylenediaminetetraacetate.

TPN more rapidly and allowing the deficient enzyme to work at its maximum rate, thereby increasing the availability of GSH which has been implicated in the maintenance of erythrocyte integrity.

106. THE LEUKEMOGENIC EFFECT OF CELL-FREE LEUKEMIC BRAIN FILTRATES AFTER PASSAGE THROUGH RESISTANT STRAINS OF MICE

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Cell-free filtrates of leukemic AKR and Swiss mouse and human brains were prepared. These filtrates were serially passed through Swiss mice in the case of the AKR mouse and human brains, and through C_3Heb mice in the case of the Swiss mice. Parallel experiments were run using the intraperitoneal and intracerebral routes of injection. At the end of 72 hours the brains of the injected animals were harvested, regardless of the route of injection, and new filtrates were prepared. The filtrate prepared from the brains of the fifth passage animals was injected intracerebrally back into groups of 20 mice of the appropriate strain (AKR when the original source was either human or AKR brain, Swiss when the original source was Swiss brain). One hundred and fifteen of 160 animals injected with filtrates whose original source was leukemic brain developed leukemia.

Heat inactivated filtrates and filtrates prepared from nonleukemic mouse and human brain were used for controls and were similarly passed. None of the 160 animals injected with heat inactivated filtrates or filtrates whose sources were nonleukemic brains developed leukemia.

Two additional control experiments were carried out. In these, tumor cell suspensions of Swiss mouse leukemia were injected intraperitoneally into C_3Heb mice at the start of the serial passage. At the same time, filtrates prepared from the brains of the same mice which served as the source of the tumor were passed through C_3Heb mice. After 5 passages, only 1 of the 40 Swiss mice injected with cell-free filtrate obtained from the tumor cell line developed leukemia, while 17 of the 40 animals injected with filtrates from the cell-free line developed leukemia. The effect of 5 serial passages is to dilute the original material to approximately 10^{-13} if one assumes 100 per cent recovery of activity in each passage. When the original cell-free filtrate is diluted beyond 10^{-5}, no activity can be demonstrated.

107. THE ELECTROCARDIOGRAPHIC DIAGNOSIS OF LEFT VENTRICULAR HYPERTROPHY IN THE PRESENCE OF LEFT BUNDLE BRANCH BLOCK

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The electrocardiographic diagnosis of left ventricular hypertrophy (LVH) in the presence of left bundle branch block (LBBB) has been considered difficult if not untenable. The purpose of this study was to compare the accuracy of the electrocardiographic diagnosis with the pathologic findings.

The electrocardiograms in the Cardiac Laboratory at the Cincinnati General Hospital were reviewed and all cases with the diagnosis of LBBB in which

an autopsy had been performed were collected. All cases with pathologic evidence of myocardial infarction were excluded. The conventional electrocardiographic criteria of high voltage for LVH were employed. Pathologic criteria for cardiac hyperthrophy included a heart weight greater than the predicted normal for body length. Specific ventricular enlargement was based upon a left wall thickness of 13 mm. or greater and a right wall thickness of 5 mm. or greater.

Utilizing the above criteria, 28 cases of LBBB were studied. At autopsy all cases had cardiac hypertrophy. Only 10 of the 28 cases showed electrocardiographic evidence of LVH. At autopsy 3 of these had pure LVH and 7 had combined hypertrophy. Eight cases of pure LVH at autopsy showed no electrocardiographic evidence of LVH.

It would appear on the basis of this study that the precise electrocardiographic diagnosis of LVH in the presence of LBBB is difficult and unreliable. The possible value of the use of the ventricular gradient as a means of distinction is considered.

108. CEREBRAL AND PERIPHERAL CARBOHYDRATE UTILIZATION DURING AMELIORATION OF HYPOGLYCEMIC SYMPTOMS BY FRUCTOSE

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Although administration of 0.1 unit per Kg. of insulin intravenously produced severe hypoglycemic symptoms in 6 subjects, an increased dose while receiving an infusion of fructose evinced only minimal symptoms when blood glucose was comparably depressed (19 to 32 mg. per cent). These observations, plus Slein's demonstration of fructose phosphorylation by brain hexokinase when glucose was absent, prompted a study of cerebral fructose utilization at low-glucose concentrations.

Fructose was infused at 1 Gm./Kg./hr. into fasting patients with normal carbohydrate metabolism. Cerebral and peripheral arteriovenous differences of fructose and glucose were determined in 14 patients, and of pyruvate and lactate in 4 patients, before and during insulin-induced hypoglycemia. Cerebral blood flows were determined in 11 patients.

Cerebral Metabolism.—Mean cerebral A-V difference for fructose was 0 ± 2 (s.d.) mg. per cent before and after induction of hypoglycemia. Despite sharp depression of arterial glucose concentrations (control, 92 ± 14 mg. per cent; during hypoglycemia, 25 ± 8 mg. per cent), cerebral A-V glucose differences also changed insignificantly (control, 10 ± 3 mg. per cent; hypoglycemia, 8 ± 4 mg. per cent). Cerebral blood flow remained constant; therefore, normal cerebral utilization of glucose persisted throughout the hypoglycemic interval (control, 6.3 ± 1.7 mg./100 Gm./min.; hypoglycemia, 6.0 ± 2.9 mg./100 Gm./min.). A-V differences of pyruvate and lactate were insignificant during both periods.

Peripheral Metabolism.—A peripheral A-V uptake of fructose was found (14 ± 5 mg. per cent); this did not change during hypoglycemia (11 ± 7 mg. per cent). The glucose A-V difference also showed no remarkable variation (control, 6 ± 5 mg. per cent; hypoglycemia, 8 ± 5 mg. per cent). Pyruvate and lactate A-V changes during hypoglycemia were minimal.

It is concluded that amelioration of hypoglycemic symptoms by fructose is not due to cerebral utilization of fructose, pyruvate, or lactate; nor to their increased peripheral extraction, with sparing of glucose for cerebral use. The

normal cerebral utilization rate for glucose (CMR_{gl}) at low arterial concentrations contrasts with Kety's observation of greatly depressed CMR_{gl} during unprotected hypoglycemia. Thus, fructose probably does serve simply as a precursor of hepatogenic glucose, thereby sustaining circulating glucose sufficiently to meet normal cerebral needs.

109. STUDIES ON THE IMMUNOLOGIC AND PHYSIOLOGIC ACTIVITIES OF ANTIHEMOPHILIC FACTOR (AHF)

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(INTRODUCED BY TIBOR J. GREENWALT, M.D.)

Hemophilia has been classically defined as a hereditary, hemorrhagic disorder characterized by a lack of an essential prethromboplastic factor in the plasma. This deficiency is determined by certain "activity" tests in which the defect is corrected by the addition of normal blood, plasma, or plasma fractions, which supposedly supply the missing factor. On the other hand, a few investigators have maintained that the abnormality is due, not to an absence of a factor, but to the presence of an inhibitor.

Utilizing a relatively pure, functionally active, human antihemophilic factor (AHF), prepared by a dextran sulfate precipitation method on Fraction I separated from platelet-poor, barium sulfate adsorbed plasma, a potent anti-AHF serum was produced in rabbits. The antiserum yielded positive precipitin reactions with dilutions of normal plasma to 1:10,000. This positive reaction disappeared completely if the antiserum was adsorbed first with the plasma of a severe hemophiliac. The anti-AHF serum also interfered with the generation of thromboplastin in normal plasma as well as with the ability of normal plasma to correct hemophilic plasma in the thromboplastin generation test. Prior absorption of the antiserum with hemophilic plasma, however, blocked its effectiveness in these actions.

Hemophilic plasma, therefore, contains a factor which is antigenically similar to that factor in normal plasma which corrects the functional defect in hemophilic plasma. If AHF is present in hemophilic plasma, one might postulate that it is not functional because:

1. It is an altered or abnormal protein. This would explain the occasional production of antibodies to AHF in hemophiliacs who have received repeated injections of normal plasma or plasma fractions.
2. It is a potentially active protein which requires an activator that is present in normal plasma.
3. It is an active protein blocked by an inhibitor which is absent in normal plasma.

110. FRACTIONATED PLASMA PROTEIN VALUES IN SUBACUTE THYROIDITIS

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(INTRODUCED BY E. PERRY MCCULLAGH, M.D.)

Stemmerman reported the occurrence of an increased concentration of serum alpha-2 globulin in 4 of 4 patients with subacute (granulomatous) thyroiditis. He suggested that this was an additional laboratory aid for the diagnosis of this disease.

The purpose of this paper is to present the results of Tiselius electrophoretic plasma protein analyses using barbital buffer in a series of 20 patients with subacute thyroiditis. Only 14 of these 20 patients had a significant elevation of the alpha-2 globulin value. Other findings were a decrease in the albumin values in 10 patients, elevation of alpha-1 globulin values in 4 patients and beta globulin values in 2 patients, and elevation of the gamma globulin in 8 patients.

The alpha-2 globulin concentration was less likely to be elevated in those patients with subacute thyroiditis whose clinical febrile course was much less acute or in those who had only one thyroid lobe involved. Thus, the value of this test as a diagnostic aid seemed to be limited to those patients with a more typical clinical febrile picture. Only 3 of 50 patients with struma lymphomatosa and none of 30 patients with nontoxic adenomatous goiter had elevated alpha-2 globulin values. Also, the presence of a number of other systemic diseases or inflammation which may produce an elevated alpha-2 globulin value must be excluded. While an elevated alpha-2 globulin may be a useful aid in differentiating subacute thyroiditis from other types of goiter, we believe clinical and other laboratory aids are probably of more value.

The elevation of the alpha-2 globulin value may be due to destruction of thyroid tissue and release of tissue protein, as observed in other types of inflammation, rather than to release of thyroglobulin alone. Recent work suggests that the elevation of gamma globulin may be due at least in part to autoantibodies to thyroglobulin in subacute thyroiditis as well as in struma lymphomatosa. Elevation of gamma globulin was present in 40 of 50 patients with struma lymphomatosa and 3 of 30 patients with nontoxic adenomatous goiter.

111. DIFFERENTIAL EXTRACTION OF NUCLEOPROTEIN FROM HUMAN LEUKOCYTES

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Laves and Thoma have reported that with the incubation of methanol fixed peripheral blood or bone marrow films in urine dialyzed against tap water, the nuclear chromatin of the neutrophilic granulocytes is lysed whereas the chromatin of all other leukocytes remains unaltered. They attribute this to a "ribonuclease complex" in urine and as a result state that the cells of the myelocytic series contain nuclear nucleotides which are predominantly of a ribose nature whereas lymphatic and monocytic cells contain desoxyribonucleotide almost exclusively.

The authors have confirmed that fresh, undialyzed urine or urine dialyzed against tap water "lyses" the nucleoprotein of the neutrophilic leukocytes without affecting that of the lymphocytes or monocytes. However, pure crystalline ribonuclease failed to produce these changes, nor did urine dialyzed against distilled water. Moreover, using yeast nucleic acid as a substrate, no significant ribonuclease activity could be demonstrated in urine. The action of urine was closely simulated by incubating the slides in 0.07 M NaCl and 0.05 M phosphate buffer solutions. The reaction was not potentiated by the addition of pure crystalline ribonuclease.

The use of salt solutions of varying concentration for the extraction of nucleoprotein is a standard procedure, and it is our impression that the dissolution of the nucleus of the granulocyte when incubated in urine is due largely to the fact that urine is basically a salt solution. The possibility of enzyme activity has not been excluded, however, and it is possible that proteolytic enzymes present in urine, particularly proteases, may play a role. The differential

effect observed could be explained on the basis of a difference in binding between the nucleic acid and the basic protein of granulocytes as compared to the mononuclear cells.

We have employed this method as a diagnostic aid in differentiating the immature cells in acute leukemia, and apparently the specificity of the reaction persists in cells as young as the blast forms. Additional data will be required to be certain of this point, but if confirmed this simple technique would provide a valuable laboratory procedure.

112. EPIDEMIOLOGIC STUDIES ON CORONARY DISEASE IN THE CHICAGO AND ILLINOIS POPULATION—RELATIONSHIP OF SEX, RACE, NATIONALITY, OCCUPATION, INCOME, AND URBAN VERSUS RURAL RESIDENCE TO MORTALITY RATES

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This study entailed utilization of the epidemiologic method to investigate problems of the etiology of coronary heart disease. Mortality data for the years 1951 and 1953 were analyzed for the middle-aged (25 to 34, 35 to 44, 45 to 54, 55 to 64) Chicago and Illinois populations stratified by sex, race, nationality, occupation, income, and urban versus rural residence. Arteriosclerotic heart disease (A.S.H.D.) death rates were several fold higher in white men than white women. Negro women rates were consistently higher than those for white women. Hence the sex differential was smaller for Negroes than for whites. Stratification of men (white and Negro) based on occupation and income yielded no gross differentials in age-specific A.S.H.D. death rates. Residents of Chicago had uniformly higher age- sex- and race-specific A.S.H.D. death rates than inhabitants of Illinois municipalities and rural areas. The Chicago foreign-born as a whole exhibited lower A.S.H.D. death rates than the native born. The foreign-born of Italian origin had the lowest rates, the Irish the highest. The Chicago foreign-born groups had uniformly higher rates than those recorded for like age-sex groups in their native countries. These epidemiologic findings bear upon basic problems currently under discussion concerning the role of diet, physical activity, socioeconomic position, endocrinology, heredity, and race in the etiology of coronary heart disease.

113. LONG CHAIN GROWTH OF GROUP A STREPTOCOCCI IN THE PRESENCE OF SERA OF PATIENTS INFECTED WITH HOMOLOGOUS TYPE ORGANISMS: A TEST FOR TYPE-SPECIFIC IMMUNITY

Gene H. Stollerman, M.D., Alan C. Siegel, M.D. (By Invitation), and Eloise E. Johnson, M.D. (By Invitation), Chicago, Ill.

Type-specific antibody to M protein appears to play a major role in immunity to group A streptococcal infection. Methods for its detection have been difficult and have hampered its extensive epidemiologic and clinical study. Recently certain strains of group A streptococci were shown to form very long chains when grown in liquid media to which rabbit antisera of homologous type were added. The reaction was shown to be specific for antibody to M protein and was simple to demonstrate.

A preliminary study was undertaken to determine the possible application of this phenomenon as a screening test for human type specific antibody. Of 28 patients who had recovered from untreated, uncomplicated type 12 streptococcal pharyngitis, the sera of 14 were shown to contain antibody against type 12 M protein by a refinement of the conventional bactericidal test. In addition, the sera of 8 patients with glomerulonephritis, which were shown to contain type 12 antibody by the bactericidal test, were selected for study. All 22 of these positive sera caused long chain growth of a selected strain of type 12 streptococci. Sera which failed to show a positive bactericidal test for type 12 antibody did not produce long chain growth.

The type specificity of the reactions was tested with strains of type 30 and type 19. These strains were shown to form long chains only in the presence of antibody to their respective homologous types of M protein. None of the human sera that contained type 12 antibody produced long chain growth of the type 19 or type 30 strains.

The results suggest that the long chain reaction may have practical value for the demonstration of human type-specific immunity to group A streptococci.

114. RESULTS OF NEEDLE BIOPSY OF THE HUMAN VENTRICULAR MYOCARDIUM

Don C. Sutton, M.D., George C. Sutton, M.D., Joshua Lynnfield, M.D. (By Invitation), and Geoffrey Kent, M.D. (By Invitation), Chicago, Ill.

Needle biopsy of the human ventricular myocardium offers the opportunity to study morphologic and histochemical changes.

Multiple needle biopsies of the right or left ventricle have been done in 17 individuals. Also, biopsies have been done immediately post mortem, at post mortem, and on dog hearts.

Long thin-walled flexible needles modified from the Terry model with inside diameters of from 0.4 to 1 mm. have been used. Percutaneous biopsies have been obtained by inserting the needle in the costo-xiphoid angle, "Larry's point," and the apex. Biopsies have also been done during pericardotomy, thoracotomy, or cardiac surgery.

It is, of course, obvious that many lesions of the myocardium are focal and that obtaining a satisfactory biopsy would be entirely fortuitous. However, diffuse lesions as those due to inflammation and nutritional disturbances are suitable for investigation. No serious complications have been encountered. The following observations will be reported: (1) Normal dog ventricular myocardium. (2) Normal human ventricular myocardium. (3) Inflammatory reaction, subpericardial in cases of tubercular pericarditis. (4) Inflammatory reaction, nonspecific, in the ventricular myocardium of a patient with sarcoidosis without clinical evidence of cardiac involvement. (5) The ventricular muscle in cases of rheumatic heart disease—biopsies were obtained at the time of cardiac surgery. (6) A case of diffuse fibrosis of the myocardium in which the biopsy was used to differentiate constrictive pericarditis, amyloid disease, and fibrosis. (7) A biopsy in a case of subendocardial fibroelastosis.

The procedure can be employed during thoracostomy or percutaneously. The tissue obtained is satisfactory for microscopic study.

115. INDICATOR-DILUTION CURVES RECORDED SIMULTANEOUSLY FROM THE VENOUS AND SYSTEMIC CIRCULATIONS

H. J. C. Swan, M.B., Ph.D., M.R.C.P. (Lond.), Irwin J. Fox, M.D. (By Invitation), André David, M.D. (By Invitation), and Earl H. Wood, M.D., Ph.D., Rochester, Minn.

Recent development of an indicator (tricarbocyanine dye II) with a peak spectral absorption in the region of 800 mμ makes possible the continuous recording in man of satisfactory dilution curves from the right side of the heart and venous circulation by photometric means. Cardiac catheters (100 cm., nos. 6 and 7 F.) have been used to permit simultaneous recording of dilution curves from various sites in the right side of the heart and from the radial artery following injection into different locations in the venous system proximal and distal to the sampling site, into locations in the left heart and into the aorta in 20 patients.

The following are among other applications such recordings permit. (1) Detection and localization of left-to-right shunts, even of very small magnitude, by demonstration of early appearing dye at or distal to the shunt following injection of indicator into a distal pulmonary artery or into the left atrium. (2) Detection and localization of regurgitation of the mitral or tricuspid valves by recognition of early appearing dye in the chamber proximal to the abnormal valve following injection distal to the valve. The competence of right heart valves in the presence of valvular regurgitation in the left side of the heart may be demonstrated by the recording of a curve of normal contour from the pulmonary artery associated with an abnormal curve recorded from the systemic system. (3) Direct measurement of pulmonary blood flow, including cases with left-to-right shunts, by the Stewart-Hamilton principle on injection of indicator into the right atrium or its tributaries and sampling from the pulmonary artery. (4) Direct demonstration of preferential shunting of the blood from the right lung in comparison to the left in the usual case of atrial septal defect. (5) Determination of shortest and mean transit times for the coronary, upper body, lower body, and other segmental circulations. Such methods, besides being of considerable practical value in diagnostic cardiac catheterization, have wide application in current cardiovascular research.

116. QUANTITATIVE PROTAMINE TITRATION TEST (HEPARIN) OF BLOOD IN ATHEROSCLEROSIS

William A. Thomas, M.D., and John H. Olwin, M.D. (By Invitation), Chicago, Ill.

In human beings there is no constant and dependable relationship between diet and blood cholesterol, or between hyperlipemia and atherosclerosis. This study, begun in 1953, was based on the clearing action of heparin, the known relation of prolonged hyperlipemia to atherosclerosis, and the development of protamine titration as a measure of heparin and heparinoid activity. The anticoagulant effect of heparin was not involved. Demonstration of lipoprotein lipase, an enzyme that regulates lipid transport quantitatively and qualitatively and is inert until activated by heparin, modified the thinking but not the procedure.

One hundred cases of known coronary or cerebral thromboses that were normotensive, nonarteriosclerotic, and nondiabetic, and atherosclerotics from peripheral vascular surgery were studied. With normal figures of 0.12 to 0.18 results showed in milligrams:

0.04	23
0.06 to 0.10	51
0.12 to 0.18	26

Forty-four hypertensive and arteriosclerotic and congestive failure cases showed:

0.10 or below	9	24.5 per cent
0.12-0.18	35	75.5 per cent

Among controls, 10 per cent or less fall below 0.10.

Mast cells are the source of heparin, and their abundance in the uterus aids in explaining female immunity to atherosclerosis until postmenopausal atrophy. Constantinides counting mast cells per cubic centimeter of myocardium reports:

Female young	351
Male young	239
Senile nonatherosclerotics	273
Senile atherosclerotics	198

Thus, atherosclerosis may result from: (1) deficiency of heparin; (2) heparinoid state incapable of activating lipase; (3) lipase deficiency; (4) lipase inert to heparin activation; or (5) abnormal lipoprotein in noncynamic state resistant to lipase modification.

There is presumably a metabolic fault in one or more of the above mechanisms in a small proportion of the population, who, when exposed to the current American diet, have an excess of low-density beta lipoproteins with resulting atherosclerosis. If the protamine titration test is a relatively accurate measure of heparin, three-fourths of this series of atherosclerosis showed deficiency. Normal values should be of aid in freeing much of the population from unwarranted dietary restrictions.

117. LACK OF SPECIFICITY OF GROWTH HORMONE ASSAY METHODS FOR DETECTION OF SOMATOTROPHIN IN BLOOD

DEVINA C. TWEED, PH.D., CLEVELAND, OHIO

(INTRODUCED BY A. CARLTON ERNSTENE, M.D.)

Methods for assay of growth hormone continue to be sought. Among those employed are (1) increase in width of tibial epiphyseal cartilage and (2) uptake of sulfur^{35} into chondroitin sulfuric acid of cartilage mucopolysaccharide.

The present study was undertaken to determine which method is preferable for assay of purified growth hormone and whether either or both could be used for detection of growth hormone in blood.

Hypophysectomized rats received a total dose of 3 ml. of whole blood plasma or varying doses of growth hormone over a 4-day period. Sulfur^{35} was administered the afternoon of the fourth day. Autopsy was performed the following morning and both assays were carried out on tibial epiphyseal cartilages obtained from the same rat. The tibia test was done as recommended by Greenspan and associates (Endocrinology **45**: 455, 1949). Sulfur^{35} was precipitated as the barium sulfate.

Results of these experiments indicate that both methods adequately measure the effects of purified growth hormone. Sulfur uptake appeared to be more sensitive in lower dose ranges (5 to 10 gamma) than increase in size of the cartilage. At higher levels of hormone concentration (50 to 125 gamma) some departure from linearity in sulfur uptake was seen.

Forty blood plasmas from patients with various endocrine disorders were assayed by these methods. The results of these tests indicate the following conclusions:

1. Plasma of patients with the same clinical syndrome does not always yield similar values when tested by these methods.
2. Plasma of hypophysectomized patients can increase sulfur uptake and epiphyseal cartilage width.
3. Plasma of active acromegalics does not always stimulate sulfur uptake nor increase cartilage width.
4. In 6 cases, sulfur uptake was increased but the tibia test was negative.
5. In 5 cases, there was no elevation in sulfur uptake but the width of the cartilage was significantly increased.
6. Repeat tests on the same patient did not always corroborate the original results.
7. In 22 cases, good correlation between the methods was obtained.

These results suggest that growth factors other than growth hormone in blood interfere with the specificity of both methods for somatotrophin assay and that sulfur uptake and general growth of epiphyseal cartilage may respond to different factors and/or to the same factors in blood to varying degrees.

118. A FIVE-YEAR STUDY OF THE ANTIBIOTIC SENSITIVITIES AND CROSS RESISTANCES OF PATHOGENIC STAPHYLOCOCCI IN A GENERAL HOSPITAL

BURTON ARMIN WAISBREN, M.S., M.D., AND CARL STRELITZER, PH.B. (BY INVITATION), MILWAUKEE, WIS.

Strains of coagulase positive Staphylococci isolated between 1952 and 1956 from 500 patients at the Milwaukee County General Hospital were studied by the tube dilution method of determining bacterial sensitivity to antibiotics. Each strain was tested against at least 10 antibiotics. The usage of antibiotics within the hospital between 1953 and 1956 was determined by examination of the daily drug requisitions from each ward of the hospital. The following information was obtained and will be presented:

1. The relative activity of the antibiotics against Staphylococci (the order is as follows: neomycin, nitrofurantoin, vancomycin, chloramphenicol, novobiocin, erythromycin, chlortetracycline, tetracycline, oxytetracycline, streptomycin, oleandomycin, bacitracin, and penicillin).
2. The antibiotics most active against strains of Staphylococci were resistant to each of the other antibiotics.
3. Statistically significant changes that occurred in antibiotic sensitivity each year and correlations between these changes and usages of antibiotics within the hospital.

4. Statistical analysis of the differences in antibiotic sensitivities of groups of Staphylococci isolated from different wards, from blood and wounds, from house staff and patients, from other hospitals, and at different times of the year. Emphasis will be on portions of these data that we have found particularly applicable to the treatment and prevention of Staphylococci infections. A program that we feel has been effective in meeting the Staphylococcus problem in our hospital will be presented.

All in all we feel that these data show that Staphylococci as a species have been little influenced by the use of antibiotics. There apparently are certain exceptions to this generalization and possible reasons for these will be discussed. Finally, the theoretical implications of our findings will be discussed and it will be pointed out that they are in harmony with modern theories of population genetics.

119. EXPERIMENTAL STUDIES WITH 5-FLUOROURACIL IN NORMAL AND LEUKEMIC RATS

HARRY A. WAISMAN, M.D., PH.D., MADISON, WIS.

The importance of uracil in the formation of nucleic acid substances has been well documented by Heidelberger and associates. The use of this substance in rats provides an opportunity to study its effect on both rat growth and on several strains of rat leukemia. The dosage of 5-fluorouracil (FU) was determined in both suckling and weanling rats. The administration of 10 mg. of fluorouracil and 25 mg. of fluorouracil to 8-day-old and 21-day-old rats gave conclusive results that the younger rats were unable to tolerate the same dosage that older rats received intraperitoneally. Hairlessness developed in suckling or nursling rats following the administration of 10 to 25 mg. of fluorouracil per kilogram body weight per day for 6 days. Death usually resulted at the higher levels. If the levels were border line and allowed the rat to recover from the toxic influence of the compound, then hair growth returned and the rat was able to survive. Evidence of toxicity in the older rats was manifested by reduction of the white count and by early death. The optimum level of 5 FU to 21-day-old rats is between 20 and 25 mg. per kilogram body weight for 7 days. Adminstration of this level to leukemic rats of the 302 and J strains provided some evidence that the substance could interfere with the "take" of leukemia. Experiments with human beings and clinical trials of the substance are now in progress.

120. CHOLESTROL PERICARDITIS

REPORT OF TWO NEW CASES WITH A REVIEW OF THE MEDICAL LITERATURE

RICHARD J. WALLYN, M.D. (BY INVITATION), AND GEORGE C. TURNBULL, M.D., CHICAGO, ILL.

Cholesterol pericarditis was first described by J. S. Alexander in 1919. Eleven cases have been reported in the world literature; and we wish to add two more patients who are typical of the syndrome.

The etiology is uncertain, one-third being idiopathic, one-third associated with hypothyroidism, and one-third being possibly of diverse etiology. The age of the patients tends to be 50 years or over (69 per cent) and the sex distribution is equal.

The characteristic clinical story is one of gradually progressive cardiac impairment due to tamponade which is usually interpreted clinically as a more ordinary form of chronic congestive heart failure. The patient insidiously develops dyspnea on exertion, orthopnea, and ankle edema.

On physical examination, the patients showed marked adynamic cardiomegaly with little or no evidence of typical tamponade. The cardiomegaly however is out of proportion to the symptoms. The electrocardiogram shows low voltage and flat T-waves.

There is partial response to medical treatment with digitalis and diuretics. Pericardiocentesis makes the differentiation from the usual forms of heart disease by producing a large volume of yellow, opalescent, "gold paint" exudate (specific gravity 1.025; protein 4.5 to 8.5 Gm. per cent). Microscopic examination of the fluid reveals innumerable cholesterol crystals plus variable numbers of leukocytes, mostly lymphocytes. Bacteriologic and mycologic studies are negative. Biopsy reveals a nonspecific, chronic pericarditis.

In the idiopathic cases, thyroxine, antitubercular and cortisone therapy have been used to no avail. Drainage of the fluid apparently causes a remission of the symptoms in some cases.

The over-all prognosis is good, with only one reported fatality from the disease itself. However recovery usually is not complete, and the heart shadow on x-ray examination remains enlarged.

The following mechanism is postulated: (1) there was an original pericardial insult that resulted in (2) pericardial granulation tissue and effusion. Thereafter the young connective tissue absorbed and synthesized cholesterol until it was deposited in and on the pericardium and (3) eventually was released into the pericardial cavity as crystals to continue chronically the inflammation and effusion.

121. THE PARADOX OF THE ANTIDESOXYRIBONUCLEASE RESPONSE IN PATIENTS WITH STREPTOCOCCAL INFECTIONS AND ACUTE RHEUMATIC FEVER

LEWIS W. WANNAMAKER, M.D., MINNEAPOLIS, MINN.

Previous investigations have indicated two paradoxes in the immunologic response to streptococcal infections. Neutralizing antibody for hyaluronidase has been observed in the majority of patients following streptococcal infection although only a small number of strains of group A streptococci (types 4 and 22) produce appreciable amounts of this enzyme in vitro. A paradox of the reverse sort has been described with streptococcal desoxyribonuclease (streptodornase). Despite the large amounts of desoxyribonuclease produced by virtually all strains of group A streptococci, few patients with streptococcal infection appear to develop antibody for this enzyme and low titers have generally been noted in patients with acute rheumatic fever.

The present report presents evidence bearing on the latter paradox. Electrophoretic studies of the extracellular products of group A streptococci have revealed the occurrence of three distinct streptococcal desoxyribonucleases. The three enzymes attack the same substrate but migrate differently on starch block electrophoresis and can be distinguished by neutralization with specific rabbit antisera. Variations in the optimal pH of activity and in inhibition by citrate have also been found. The three streptococcal desoxyribonucleases are designated Dnase A, B, and C.

The demonstration of three serologically distinct desoxyribonucleases of group A streptococci appears to explain the paradoxical findings previously noted. Studies of sera from 38 patients with acute rheumatic fever and of paired acute and convalescent sera from 48 patients with streptococcal infections indicate that the antibody response for each of the enzymes is independent of the other two. Since Dnases A and C are elaborated by relatively few strains of group A streptococci, an immunologic response to these two enzymes is infrequently observed. On the other hand, a rise in antibody to Dnase B can be demonstrated in approximately 80 per cent of patients following streptococcal infection and elevated titers are present in almost all patients with acute rheumatic fever. This antibody test (anti-Dnase B) is useful in conjunction with the antistreptolysin O test as an indication of the probability of recent streptococcal infection and has advantages over other commonly used secondary tests (antihyaluronidase and antistreptokinase).

122. TUMOR INHIBITING EFFECTS DERIVED FROM AN ACTIVE PRINCIPLE OF GARLIC (ALLIUM SATIVUM)

Austin S. Weisberger, M.D., and Jack Pensky, Ph.D. (By Invitation),
Cleveland, Ohio

Extracts of garlic (allium sativum) contain a powerful bactericidal agent. This compound, allyl allylthiosulfinate (allicin), is formed in crushed garlic bulbs by the action of an enzyme (alliinase) on a substrate, s-allyl L-cysteine sulfoxide. Allicin has been shown to react with cysteine and to inhibit several sulfhydryl enzymes without affecting many other enzymes. Although most studies on allicin have been concerned with its bacteriostatic properties, its reactivity with sulfrydryl groups suggests that it may have inhibitory effects on leukemic and neoplastic cells. Accordingly, the effect of such compounds (R-SO-S-R) on the growth of sarcoma 180 ascites tumors in mice was studied. Since the naturally occurring allyl allylthiosulfinate is unstable, the more stable ethyl analogue was used. The enzyme alliinase was prepared from garlic bulbs and was allowed to react with s-ethyl L-cysteine sulfoxide to form ethyl ethylthiosulfinate (ethios).

Gain in weight and length of survival were taken as an index of the amount of ascites formed and the degree of malignancy induced. Preincubation of tumor cells with saline prior to injection was uniformly followed by rapid weight gain and death within 16 days in control mice. Similar results were obtained when tumor cells were preincubated with either enzyme or substrate. However, when tumor cells were preincubated with a solution in which the enzyme and substrate had been allowed to react, no growth of ascites tumor occurred and there was no mortality in animals observed for 6 months. Similar results were obtained with synthetically prepared ethios. Intravenous injection of ethios into mice previously inoculated with tumor cells delayed the onset of maligant ascites and in some instances completely prevented ascites formation and death.

The effects of ethios on incorporation of S^{35} L-cysteine by leukemic leukocytes in vitro was also studied. Following incubation with increasing amounts of ethios, there was a progressive decrease in S^{35} uptake. With 2.5 μm of ethios per milliliter blood the uptake of S^{35} was reduced to 10 per cent of control values.

These effects by an agent which inactivates sulfhydryl groups are further suggestive that sulfhydryl metabolism may have an important role in leukemia and neoplasia.

123. STUDIES ON THE ACID-BASE BALANCE OF HYPERTENSIVE PATIENTS

JOHN M. WELLER, M.D., ANN ARBOR, MICH.

The acid-base balance and plasma electrolytes of 12 normotensive subjects and 17 patients with essential hypertension have been studied. None of the hypertensive subjects was receiving antihypertensive drugs. After 30 minutes recumbency arterialized, heparinized, venous blood was withdrawn from the heated arm and the plasma separated anaerobically. Two blood pressure reading were taken before and following venesection. The mean of these systolic and diastolic pressures was used for classifying the subjects into two groups; the normotensive group contained individuals with mean blood pressures below, and the hypertensive group those above 120 mm. Hg. The group mean of the mean blood pressures of the normotensive subjects was 99 mm. Hg and that of the hypertensive patients 160 mm. Hg.

A study of the plasma electrolytes of these patients disclosed a slightly greater sodium concentration (146 mEq./L.) in the hypertensive than in the normotensive group (143 mEq./L.). This difference in plasma sodium concentration which previously has been reported by others was accentuated when the plasma sodium was expressed as concentration per liter of plasma water.

The mean plasma pH of the hypertensive group was slightly lower (7.30) than that of the normal subjects (7.33); plasma carbon dioxide contents were similar in both groups; the calculated plasma buffer base concentration was slightly lower in the hypertensive than in the normotensive group (45.6 as compared with 47.0 mEq./L., respectively), and the pCO_2 was higher in hypertensive than in normotensive patients (51.9 as compared with 48.2 mm. Hg). These findings tend to confirm the reported association of respiratory acidosis with essential hypertension. Further studies are now in progress to determine the effect on this respiratory acidosis of lowering the blood pressure of hypertensive patients by treatment with antihypertensive drugs.

124. ADRENOCORTICAL FUNCTION IN GOUTY ARTHRITICS

G. M. WILSON, JR., M.D. (BY INVITATION), RICHMOND W. SMITH, JR., M.D., JOHN W. SIGLER, M.D. (BY INVITATION), AND DWIGHT C. ENSIGN, M.D., DETROIT, MICH.

The uricosuric and antigout effects of adrenocorticosteroids are generally accepted. Earlier studies of gouty arthritics have failed by indirect methods to demonstrate abnormal adrenocortical function. As a more specific measure of this function, the diurnal and corticotropin-induced changes in plasma levels of free 17-hydroxycorticosteroids (Porter-Bush Method) have been followed. Observations were made in 12 patients during the subacute and resolving steps of arthritis attacks.

The mean levels at 8 A.M., 12 noon, and 4 P.M. were 25.6, 18.1, and 16.9 μg per cent, respectively. The mean values in the control group (11 healthy adults) were 20.9, 17.0, and 15.3 μg per cent, respectively. Analysis of these data for control values and degrees of diurnal fall demonstrates no significant difference. After 10 units of subcutaneous corticotropin-gel the mean level in 8 gouty subjects rose in 4 hours from 16.7 to 33.0 μg per cent. In the 11 controls the mean level increased from 19.3 to 26.5 μg per cent. Although the increment of change in the gouty patients appears considerably greater than

in the normal, the difference between the initial and final 4-hour values was not significant. Plasma levels of free 17-hydroxycorticosteroids were often elevated during the active arthritis in gouty subjects.

These observations support previous evidence of normal adrenocortical function in gouty arthritics.

125. PRIMARY RETENTION OF WATER IN HEPATIC COMA

PHILIP C. YOUNG, M.D. (BY INVITATION), HARVEY C. KNOWLES, JR., M.D., AND LEON SCHIFF, M.D., CINCINNATI, OHIO

It has been shown by acute water loading that water intolerance occurs in liver disease. The present study deals with water and sodium balance in the course of hepatic coma.

Observations were made on 10 patients with hepatic coma in whom water and Na balance were estimated for periods of 4 to 13 days. The patients were divided in 3 groups. In the first group of 3 there was no nitrogen retention and all recovered. Two were not edematous and remained in water and Na balance; while the third was edematous and lost approximately 2 L. of water over and above that associated with Na diuresis during cortisone therapy. Three patients comprising the second group were edematous and had nitrogen retention but had normal kidneys at necropsy. Urinary volume was normal. Positive water balance occurred ranging from 4 to 8 L. but was unassociated with Na retention. In the third group of 4 patients, nitrogen retention occurred and renal tubulorhexic lesions were found at necropsy. There was progressive oliguria in 3. In the fourth patient, despite normal urinary volume, there was an accumulation of 4 L. of water in excess of that accounted for by Na retention. Hyponatremia was found in all, but its degree was unassociated with water retention.

The findings indicate that a primary retention of water may occur in the course of hepatic coma. This water retention was associated with renal failure in some instances while there was no impairment of renal function in others. The mechanism of water retention in the latter is unexplained.

126. LIPID MOBILIZER HORMONE IN HYPERCHOLESTEROLEMIC STATES AND IN SURGICAL STRESS

CHRIS J. D. ZARAFONETIS, M.D., JOSEPH SEIFTER, M.D. (BY INVITATION), DAVID BAEDER, PH.D. (BY INVITATION), AND JOHN KALAS, M.D. (BY INVITATION), PHILADELPHIA, PA., AND RADNOR, PA.

Lipid mobilizer (LM) is a naturally occurring hormone which has been isolated from the plasma of several animal species, including man, and from hog posterior pituitary. Intravenous administration of LM into fasting patients elicits a marked increase in plasma cholesterol, fatty acids, and lipid phosphorus. Furthermore, the concentration of LM in plasma has been shown in animals to be increased by conditions of stress, including starvation, and by the administration of cortisone.

These and other observations suggested that certain hypercholesterolemic states in man may, in fact, reflect excess LM activity. It was also postulated that the stress of an operative procedure might cause an acute LM release, followed by a rise in plasma lipids. The present report is concerned with studies undertaken to test these hypotheses.

Fasting blood specimens were obtained from appropriate patients for LM assay: The plasma was dialyzed through viscose No-jax casing in a volume of sterile deionized water 4 times greater than that of the plasma. After 24 hours of dialysis, the dialysate was freeze-dried for 18 hours. The test material was injected intravenously at doses of 1, 5, and 10 mg./Kg. into fasted sensitized rats. The rats were sacrificed two hours later and blood samples obtained from the heart were analyzed for changes in lipids. By this method, it was found that the hypercholesterolemic patients tested showed much greater concentrations of LM in their plasmas than is found in subjects with normal plasma cholesterol values. This was true for several members of a family with hypercholesterolemic xanthomatosis and in individual patients with hypercholesterolemia. Similar results were obtained in preliminary tests on patients with hypercholesterolemia of pregnancy, and of nephrosis.

Studies were also performed on plasmas obtained from patients before and 4 hours after operation. In a majority of instances there was an increase in LM concentration and an accompanying rise in plasma lipids in the postoperation specimens.

Thus, increased concentration of plasma LM was induced by surgical stress as well as found to be associated with both primary and secondary hypercholesterolemic states, thereby lending support to the above indicated hypotheses.

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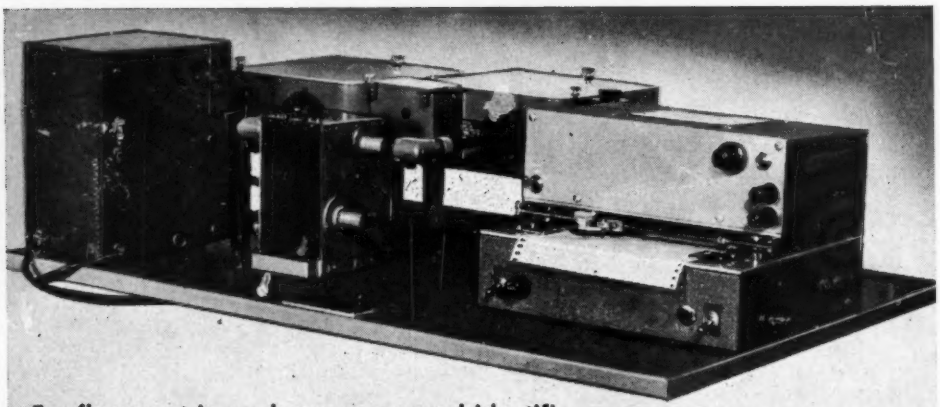

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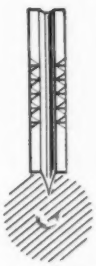


NEW B-D STERILE DISPOSABLE BLOOD LANCET

unique "gape-incision" gives you...

adequate blood flow
The new B-D LANCET produces a half-round incision that tends to pout or "gape"— avoids premature closure— clotting delayed

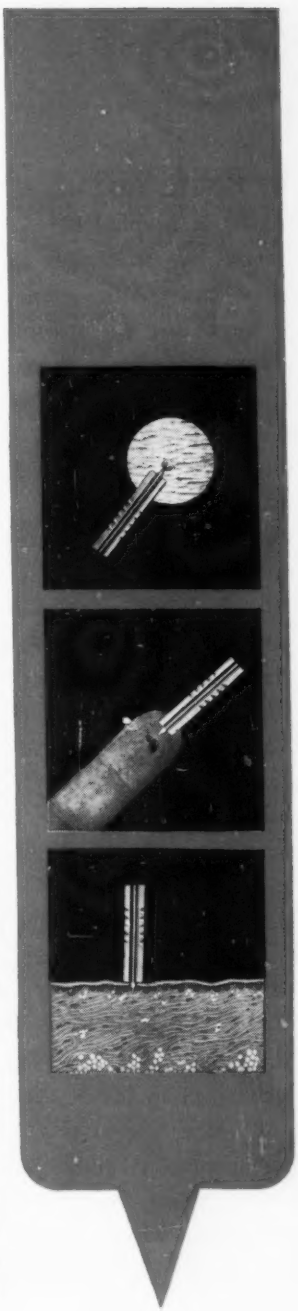
result—an easier and immediate yield of an adequate flow of blood.

minimal dilution
The "gape-incision" of the B-D LANCET makes "milking" of the finger tip unnecessary— just a gentle pressure.

result—a minimal dilution of the blood by tissue fluids—a truer specimen of the patient's blood

controlled penetration
Side flanges of the B-D LANCET automatically control depth of penetration. The angle and length of the point ensure that incision is in the region of densest capillary supply

result—easier penetration— fewer tactile corpuscles traumatized—minimal pain.



- stainless steel—very thin, yet rigid
- sterile—ready for immediate use
- hermetically sealed in preformed foil
- translucent top for positive point location
- conveniently packaged in 100's and 250's

BECTON, DICKINSON AND COMPANY, RUTHERFORD, N. J.
B-D, T.M. REG. U.S. PAT. OFF. 49357

B-D

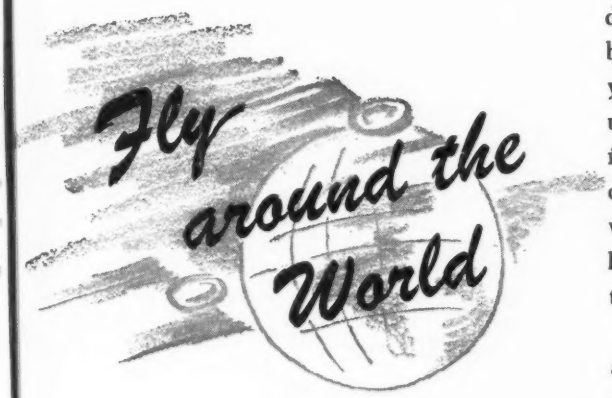
UP UNTIL 1952 one of the greatest problems in hospital blood bank laboratories was how to identify abnormal antibodies—easily. Although new techniques were revealing the presence of more and more antibodies in incompatible crossmatches, transfusion reactions and hemolytic disease, in most cases, the antibodies could not be identified for lack of a test panel.

Then Knickerbocker introduced their revolutionary professional panel of Group O cells. It fairly rocked blood grouping centers across the country. Antibodies that previously had to be sent out to specialists for diagnosis were now identified at home and at once. This technological marvel was called PANOCELL® (from *pan*el of *O cells*), and a good name, too.

We think of PANOCELL as a tool for the precise identification of unknown antibodies in serum. In contrast to Anti-A and Anti-B agglutinins which are used to identify the unknown red cells, PANOCELL provides the red cells used to identify the unknown antibodies. It is a panel of eight especially selected Group O cells, each of which represents a different Rh-Hr combination. In addition, each cell is definitively typed for 23 different blood group antigens including such systems as Duffy, Kell, Lewis, Kidd, MNS, P, etc., which are charted on an accompanying masterlist.

But that wasn't all. In order to ship the red cell suspensions through the mails, Knickerbocker had to solve the problem of hemolysis. And this great pioneering organization met the challenge by formulating a new and original preservative solution[1], the now famous "Knickerbocker cocktail." So successful is this recipe that PANOCELL has become a world traveller and safely flies over the length and breadth of the United States, across the Atlantic Ocean to England, across the Pacific Ocean to Hawaii, and across and around the world to far-off Johannesburg, South Africa. Never before did red cell suspensions fly so far and arrive so well.

REPORT
NUMBER 4



But how, a layman might well ask, does PANOCELL help the hospital laboratory? The answer is that the laboratory technologist, using PANOCELL and the same routine techniques that detected the antibody abnormality, may now precisely and easily identify it. The result is an exact clinical diagnosis in place of an unknown. And the diagnosis may be confirmed by having the reactions checked by Knickerbocker themselves, who do this work at no fee.

What a wonderful tool PANOCELL really is. In one instance a completely new antibody, called anti-V[2], was discovered by using PANOCELL. Since that time 12 different examples of anti-V have been found by the panel subscribers. There are now 83 blood centers in every section of the United States who regularly do their own independent blood studies because of PANOCELL. Day after day, year in and year out, this elite group picks up antibodies and identifies them, explaining hemolytic disease and preventing incompatible transfusions. What a wonderful impetus PANOCELL and Knickerbocker have given to the art and to the profession.

Knickerbocker has bound into a folder a small collection of descriptive literature and reprints about PANOCELL which interested readers of this report would probably like to read. There is also a long list of famous typing serums, blood group specific substances that are separate, and new laboratory controls like PROTROL, the world's most successful human prothrombin control. So will you write to Knickerbocker, 300 West 43rd Street, New York City, and ask them to send you the "PANOCELL Folder." And also please accept this standing invitation to make use of the consultation services provided at no fee to the profession by Knickerbocker. Remember Knickerbocker makes its products and services available directly to you to assure freshness and good service.

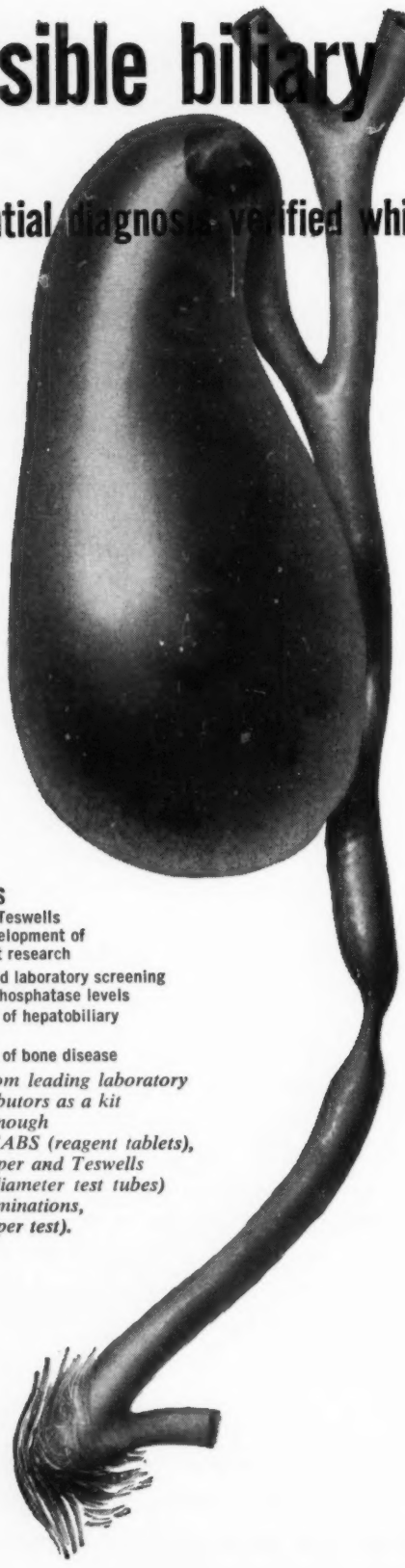
1. J. A. M. A. 158:971 (July) 1955
2. J. A. M. A. 159:247 (Sept.) 1955

Knickerbocker

THIS REPORT IS THE FOURTH OF A SERIES.

possible biliary stones?

differential diagnosis verified while your patient waits...



Phosphatabs* (alkaline)

with Teswells*

For alkaline phosphatase screening when you suspect:

- common duct stones
- biliary stasis
- stricture of the common bile duct
- fibrosis of the sphincter of Oddi

Diagnosis of impaired biliary drainage can now be verified in less than 30 minutes, through a new procedure so simple it can be performed on serum or plasma without special equipment and by untrained personnel — PHOSPHATABS for alkaline phosphatase screening.

Serum alkaline phosphatase is excreted through the bile. High alkaline phosphatase levels are evidence of impaired biliary drainage.[1]

Traditional methods of determining phosphatase levels are too complicated to make their office use practical. Now alkaline phosphatase determinations can be made semiquantitatively in the office — easily, accurately.

THE USE OF PHOSPHATABS CAN BE TAUGHT IN 10 MINUTES. RESULTS CAN BE READ IN AS LITTLE AS 12 MINUTES, WITHOUT SPECIAL INSTRUMENTS.

To use: 1. Place one tablet (PHOSPHATABS) in the special test tube. **2.** Add 4 drops of serum or plasma. **3.** Crush tablet with wooden applicator stick. **4.** Let stand 12-30 minutes, depending on room temperature (determine time from table in PHOSPHATABS package insert). **5.** Add one drop of PHOSPHATABS color developer. **6.** Compare color with color chart.

1. Gibbons, T. C.: J.A.M.A. *164*:22 (May 4) 1957.

PHOSPHATABS
(alkaline) with Teswells
an original development of
Warner-Chilcott research

- for office and laboratory screening of alkaline phosphatase levels
- in diagnosis of hepatobiliary dysfunction
- in diagnosis of bone disease

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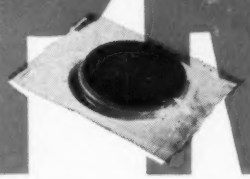

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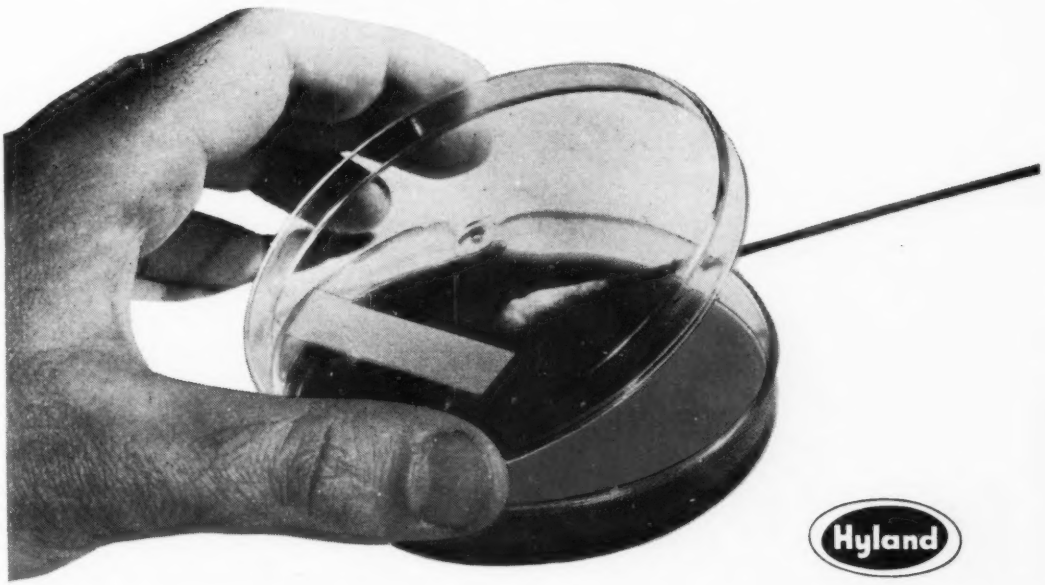

it's ready to use – just streak it

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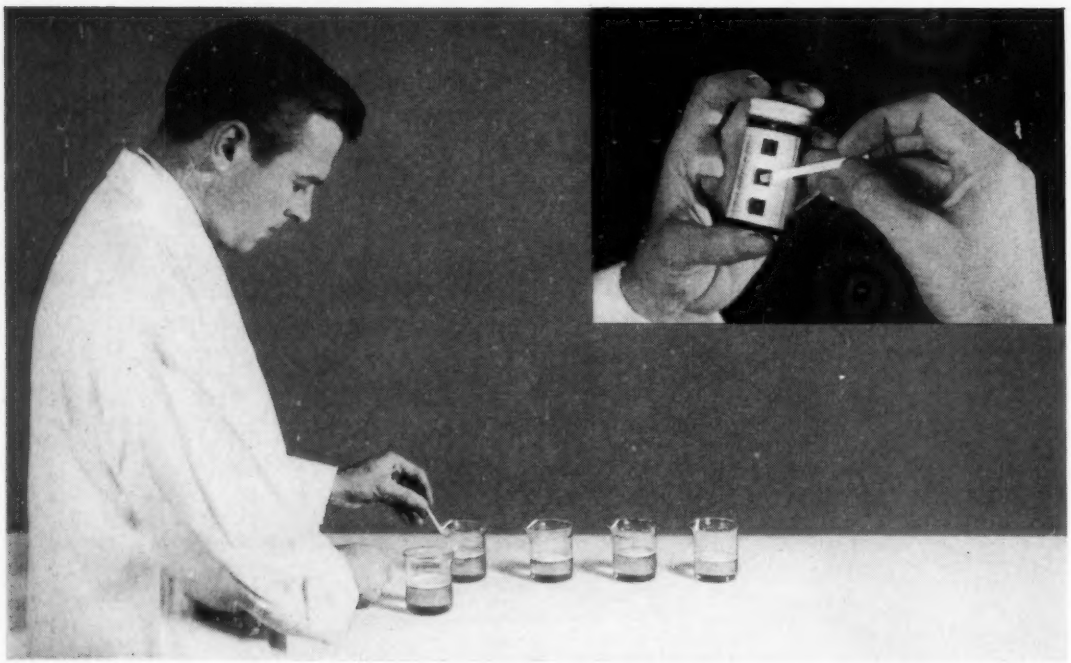

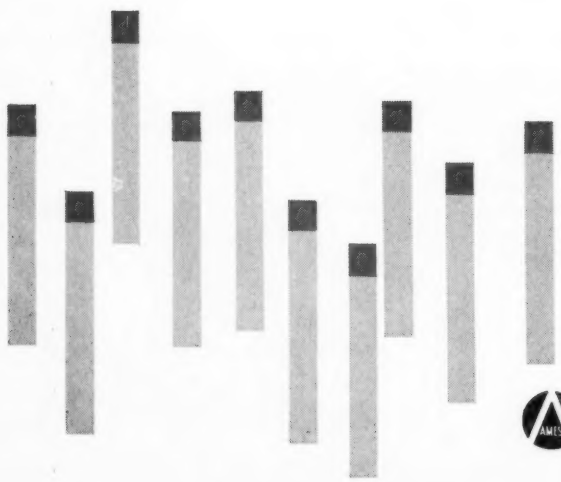

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sensitive greater relative sensitivity to acetoacetic acid—the more reactive ketone

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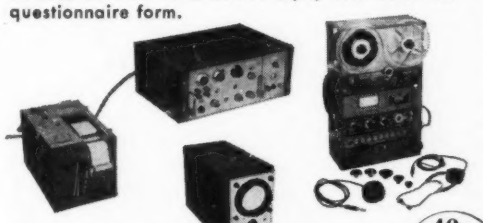

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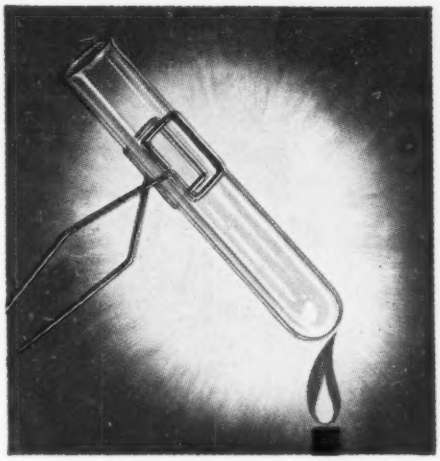

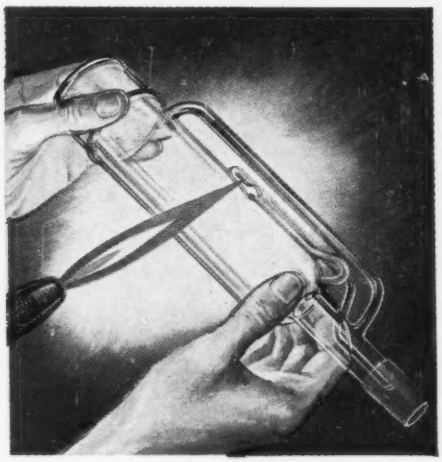

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With the Editorial Assistance of

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Howard University, Washington, D.C., Editor of the Journal, Experimental Parasitology

CHANGING populations, especially migration from endemic areas, and greatly increased world travel, have contributed much toward the rising awareness of parasites and tropical diseases in the United States. Northern and eastern areas are not exempt from this potential infiltration. Encouragement lies in the significant modern concept which has been introduced in this edition.

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(Published August, 1957)

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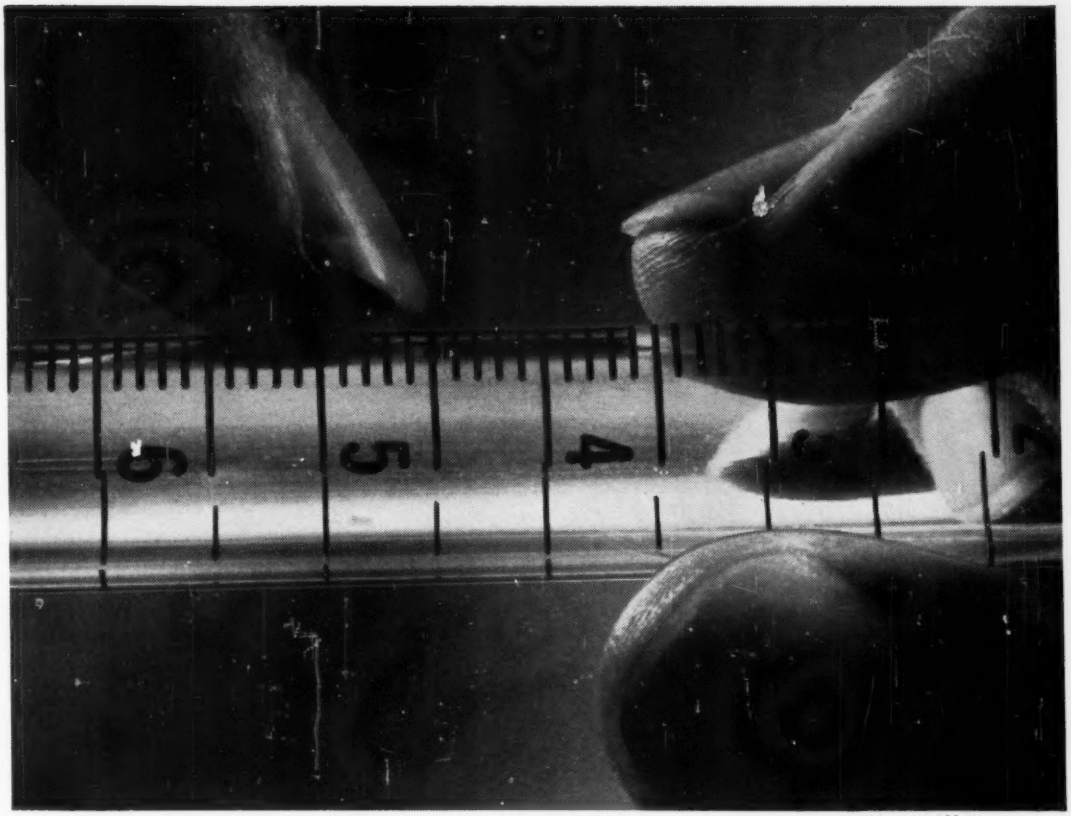

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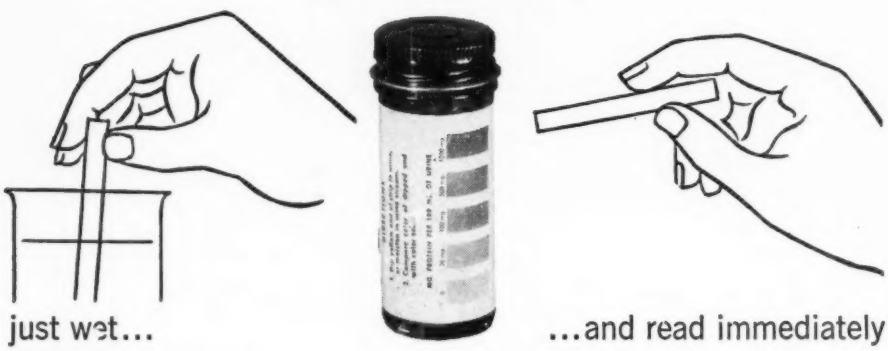

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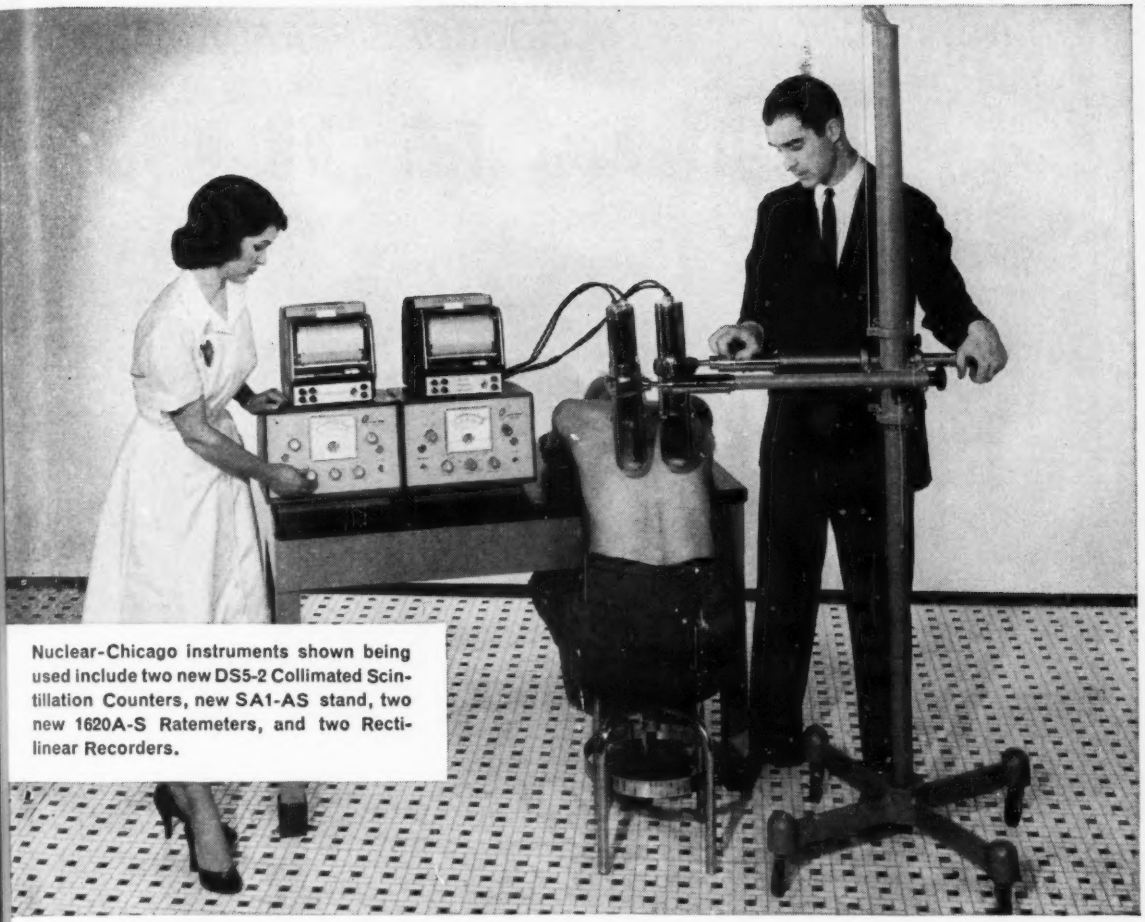

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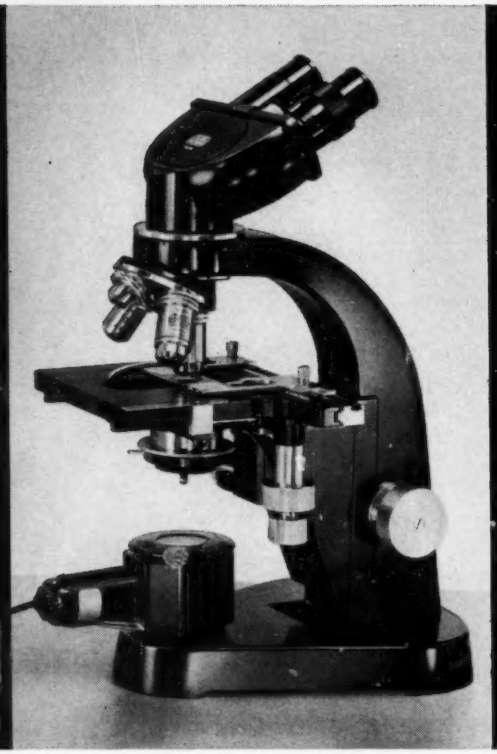

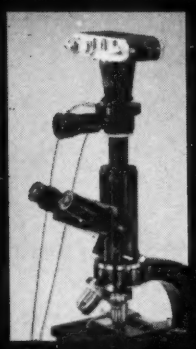

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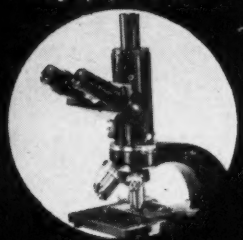

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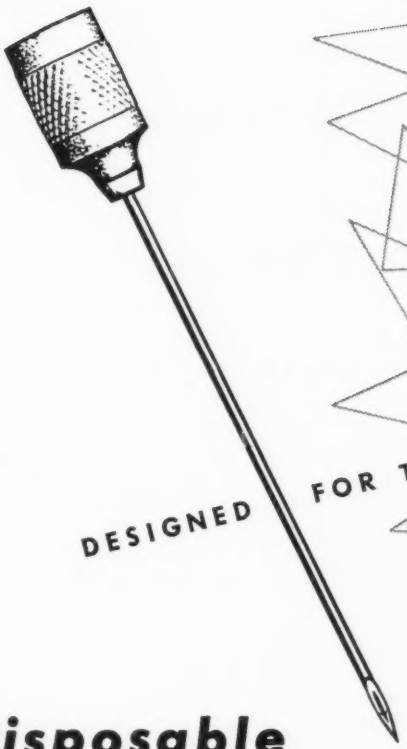

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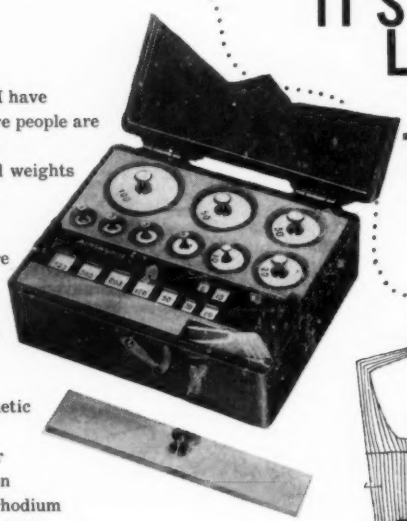

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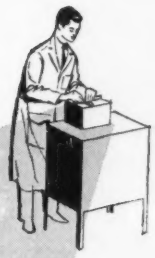

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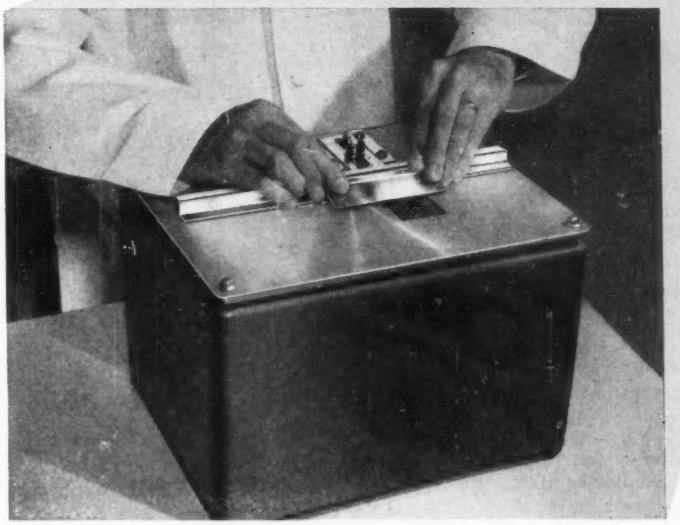

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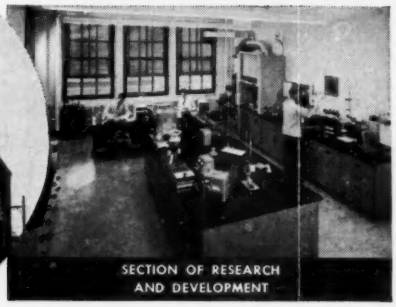

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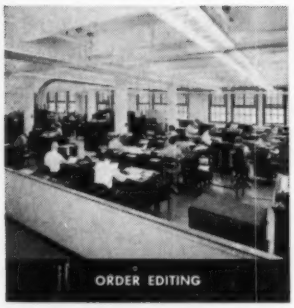

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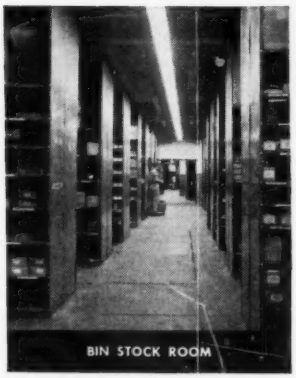

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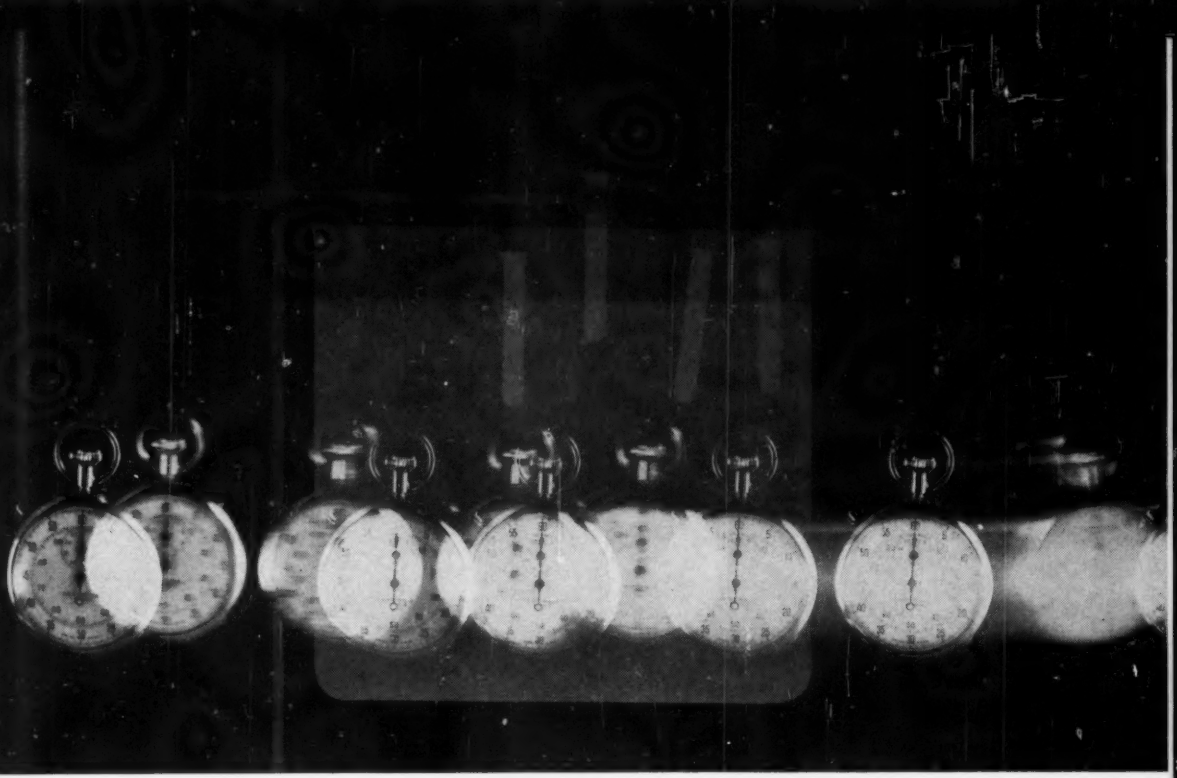

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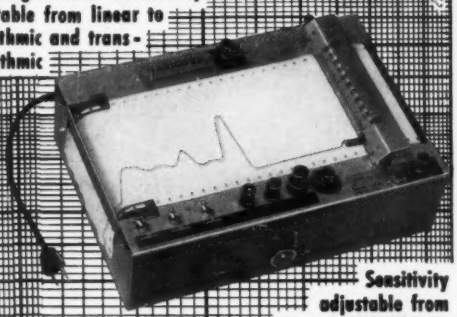

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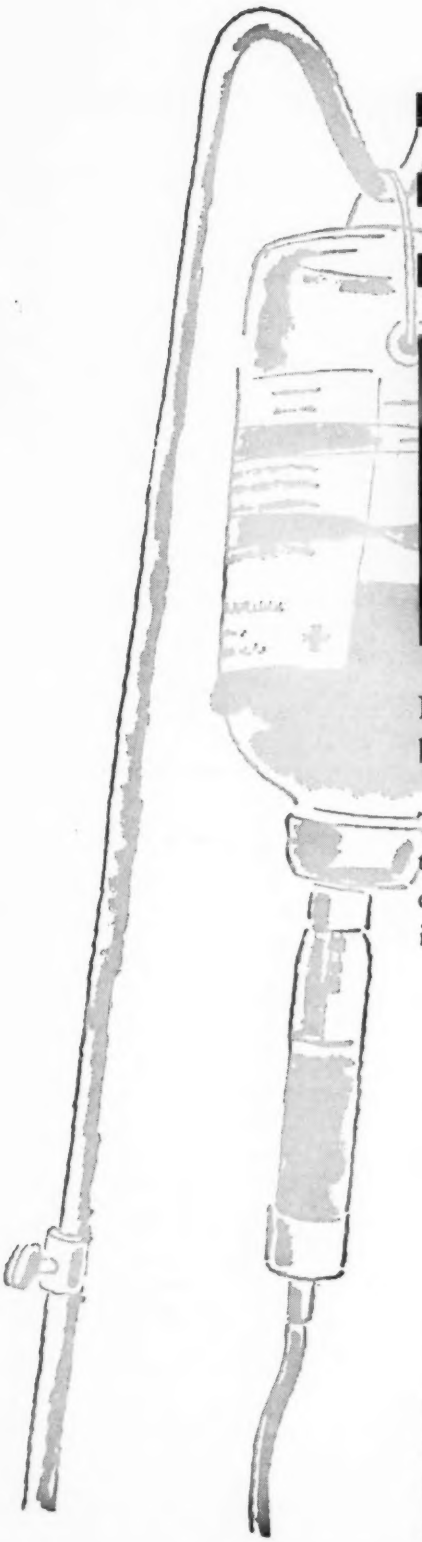

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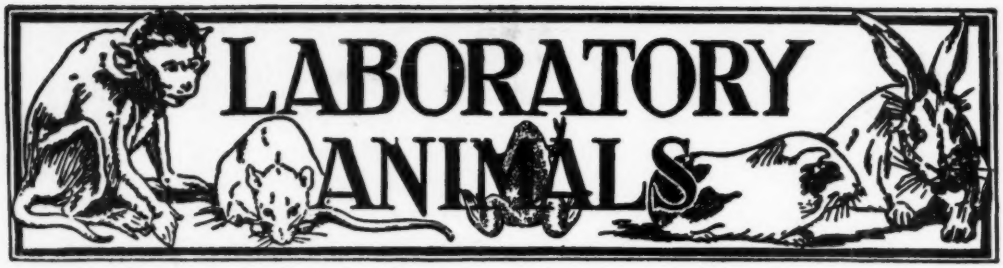

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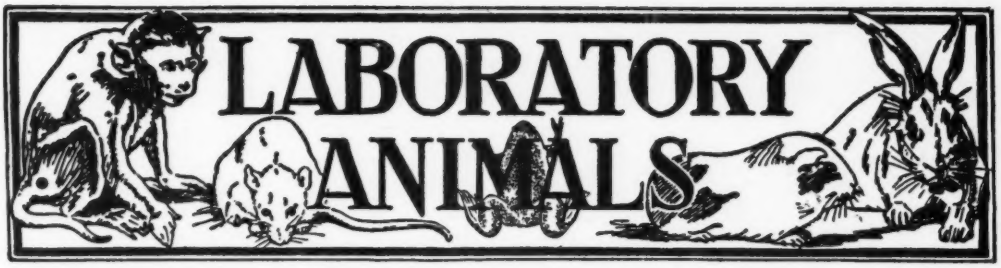

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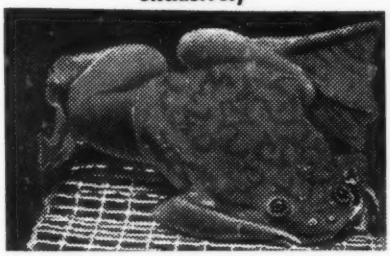

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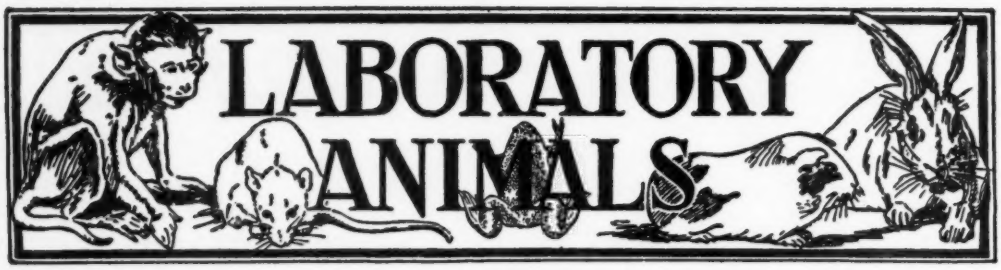

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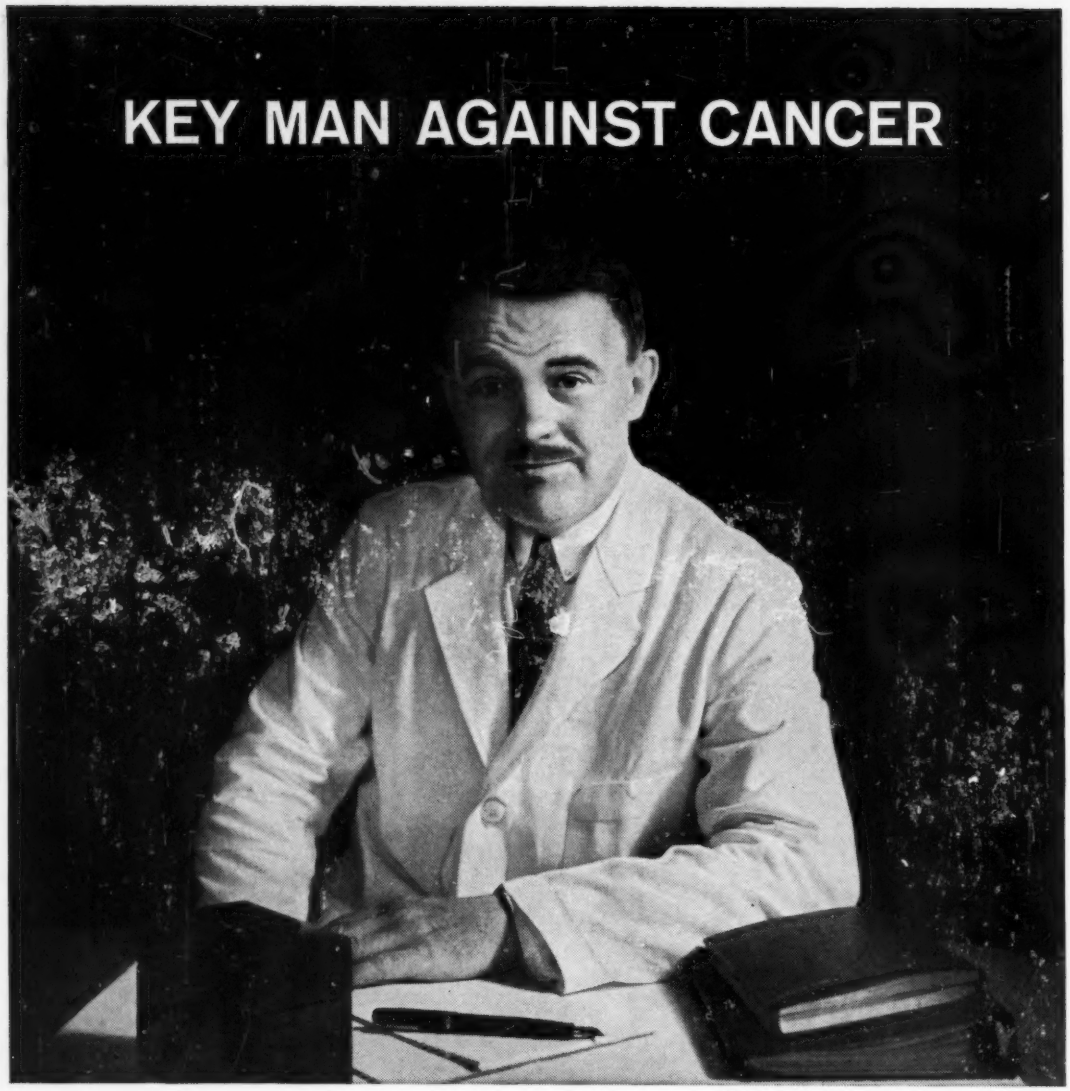

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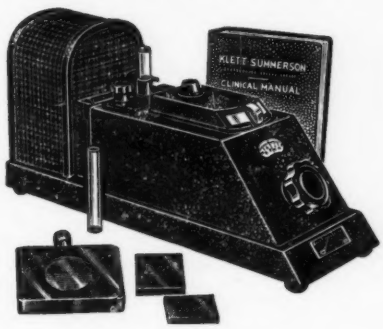

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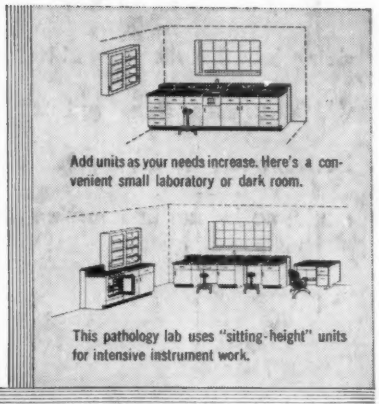

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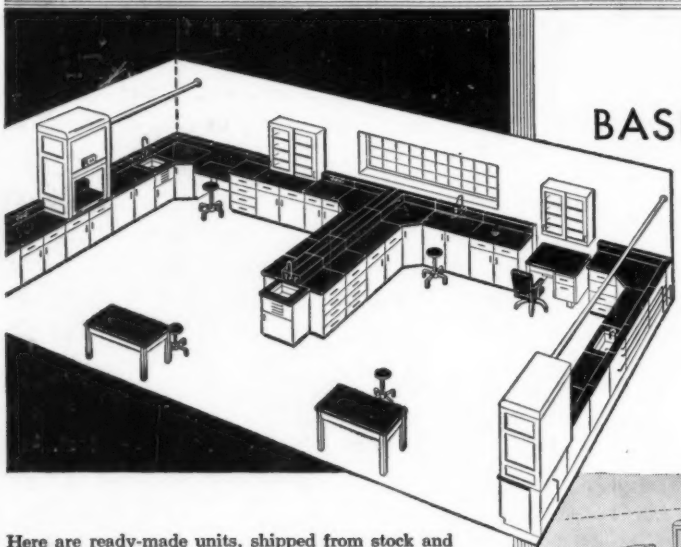

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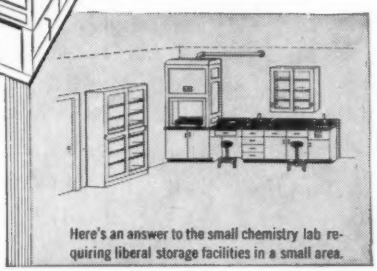

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